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OENOTHERA LAMARCKIANA MUT. VELUTINA

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(WITH PLATE 1)

One of the rarest mutations of *Oenothera Lamarckiana* is that in which those qualities are changed which constitute the characters of the twin hybrids *laeta* and *velutina*.[1] This is the more striking because in hybrid combinations these twins appear often and, as it seems, easily. But in the same way many other mutations, which apparently might reasonably be expected, have not as yet been observed and therefore must be assumed to be at least very rare. Why some mutations are common and others rare is still an open question.

From the behavior of the twin hybrids in crosses we may deduce that the mutant *velutina* must be in the main recessive to the parent species, and that the mutant *laeta* should be dominant over the *velutina*. If there were but one character involved, this would mean that the mutant *laeta* must be externally like *O. Lamarckiana*, and the same conclusion would have to be admitted if there were more characters indissolubly bound together. This being granted, the *laeta* could, of course, never be expected to appear as a mutant.

For some years, however, my cultures tend to show that the mutations observed in the group of the Oenotheras are far more compound phenomena than I was formerly inclined to assume. This also seems to be the case with the splittings which so often

[1] DE VRIES, HUGO, On twin hybrids. BOT. GAZ. 44:401–407. 1907.

occur after hybridization, and especially with those which appear in the first hybrid generation. If we apply this view to the twin hybrids *laeta* and *velutina*, the possibility is at once revealed that the components of this group of characters might not always be so indissolubly connected, and that some deviating combination of these qualities might still produce a mutant *laeta*, different from the type of *Lamarckiana*. As a matter of fact, a pure and complete mutant *velutina* has appeared in my cultures, but a *laeta* has never been seen as a mutant. In crossing this *velutina* with the parent species, however, twin hybrids arose, one of which may be designated as *laeta*, as will be shown later.

The question whether the characters of mutants and of hybrids among the Oenotheras are single or built up of a less or greater number of theoretically independent units now seems to be one of principal interest to me. Until now, however, the analysis of the qualities of the twin hybrids *laeta* and *velutina* has been difficult and unreliable on account of the presence of the hereditary qualities of their other parent. We can make inferences from these by comparing the twins issued from the crosses of different species with *O. Lamarckiana*, but we can hardly expect to get a complete analysis in this way. The mutant *velutina* is free from these specific admixtures, and therefore may afford a better material for experiments in this direction. I intend to study it from this point of view by means of a number of crosses, most of which I made during the summer of 1915, and shall give here only a description of the mutant itself, and of those hybridizations which give proof of its right to the name of *velutina*.

In order to avoid the confusion which might easily arise from the similarity of the names *O. Lamarckiana* hyb. *velutina* and *O. Lamarckiana* mut. *velutina*, I will give a synonym to the latter and call it *O. Lamarckiana* mut. *blandina*, or briefly *O. blandina*. In descriptions the use of this latter term will be obviously the easier. *O. blandina* has throughout its life and in all its organs a paler tinge than *O. Lamarckiana*.

O. blandina is, in all respects and at every stage of its evolution, strikingly different from *O. Lamarckiana* and easily recognizable. Its marks become visible with its very first leaves, when still in the

seed pans, about 4–6 weeks after sowing. At the time when the seedlings must be planted in larger boxes, their marks are fully reliable, even in hybrid mixtures. Their leaves are narrow and a pale yellowish green, whereas those of *O. Lamarckiana* are broad and a deeper green. As the rosettes increase the number and the size of their leaves, the differences between the two types are seen to increase also, and after some weeks more, when the young plants are ripe for transplanting into the garden, *O. blandina* is clearly a type of its own and can easily be counted off in the mixtures. In May the rosettes of *O. Lamarckiana* gradually become very stout, but those of *O. blandina* remain slender. The leaves are so narrow as not to touch each other regularly nor to cover the ground between them. They resemble those of *O. rubrinervis*, but lack the brittleness, the typical bending of the petiole and blade, and the specific color of this form. Moreover, they show a high degree of fluctuating variability in their color. This is always gray, on account of the hairiness of the surface, but it varies between a normal green and a more or less pale and yellowish tinge. The paler they are, the poorer they are in chlorophyll, and therefore the palest individuals soon begin to show a slower growth. They stay behind the others the more, the paler they are. These differences increase if the culture is densely planted, and even at the time of flowering the paler individuals may be seen to be much weaker and shorter than the others. In ordinary cultures, however, when the plants are at a distance of 20 cm. or more from their next neighbors, and grown in a well manured soil, these differences gradually disappear and are no longer visible when the first flowers open. In order to fertilize pale individuals beside the green ones, I had to mark them in early youth. From the self-fertilized seeds of the green ones the seedlings are on the average less pale than those of the paler parents, but the difference, although obvious and unmistakable on the beds in springtime, soon disappears as the summer begins. From time to time there is even a partial variability, such as, for instance, a green branch on a stem with pale leaves. This shows that there is no racial difference between the green individuals and the pale ones, even as in the cases of *Oenothera* (*Lamarckiana*×*atrovirens*) *gracilis* and of *O.* (*Lamarckiana*×

Hookeri) *velutina*.[2] Since making these experiments (1912–1913) I have cultivated *O. blandina* so as to reduce the paleness of its leaves to the first youth of the rosettes, and to have no diminution of the individual strength of my seed-bearing specimens on account of it.

At the time of flowering the plants are much more slender than those of *O. Lamarckiana*, and are in their main features very much like the *velutina* hybrids of *O. biennis*×*Lamarckiana* and of *O. Lamarckiana*×*O. biennis Chicago*, and especially like the latter, with which in some instances they can easily be confounded. The leaves on the stem are narrow, reaching about two-thirds the breadth of those of *Lamarckiana* if compared by equal length. In the beginning they are folded along their midvein, but later they become flattened, and this curious character may then be seen only in the bracts of the inflorescence. The bubbles, which are so characteristic of the leaf blades of *O. Lamarckiana*, are absent in the mutant. I shall designate this lack of bubbles by the term "smooth."

The flowers of *O. blandina* are cup-shaped, whereas those of the parent species are more or less quadrangular. The size is the same, the color is as bright, and the stamens show no marked difference; the supply of pollen is very large in both cases. The stigma is widely spread out above the anthers, the distance being even somewhat larger in the mutant (1 cm. against 0.5 cm.). The flower buds are almost twice as thick in the mutant, more regularly and more deeply colored with red brown lines and spots, and much more hairy. This color and this hairiness extend over the tube of the flower and the ovary, and in a less degree over the top of the stem and the young bracts. The small free tips at the top of the flower bud are thick in *O. blandina* but thin in *O. Lamarckiana*. The differences of the fruits are small, except for the hairiness. The most striking character of *O. blandina*, however, is seen at the end of the flowering period, when the spikes are long and the lower fruits begin to ripen. At that period the spikes are very slender, with few fruits and long internodes, whereas on the spike of *O. Lamarckiana* the fruits are densely crowded. I counted the fruits

[2] Gruppenweise Artbildung. Berlin, 1913, p. 164, where *O. atrovirens* still bears the name of *O. cruciata* (*fig. 73*); and p. 116, *fig. 46*, for the twin hybrids of *O. Hookeri*.

on a length of half a meter in the middle part of the spike, at the end of September, and found 30 of them on *O. blandina*, but 75 on *O. Lamarckiana*, both on very vigorous annual specimens. From this the internodes of the spike are 1.7 against 0.7 cm., or more than twice as long as those of the parent species. The average numbers of flowers which open on a spike during an evening are inversely proportional to these figures. For many crosses I have castrated five successive flower buds of *O. Lamarckiana* on one day and pollinated them the next day, whereas the crossing of 5 flowers on a spike of *O. blandina* usually lasts 4 or more days, which makes quite a difference in the technical work.

Of course, there are a number of distinguishing points of less value, but their description would remain vague unless strict averages could be given; and since all characters are more or less dependent upon the conditions of soil and culture, it is doubtful whether even averages would be reliable. I shall return to this point in the description of the hybrids.

In comparing this description with that given in my book on *Gruppenweise Artbildung* for *O.* (*Lamarckiana*×*O. biennis Chicago*) *velutina*, it will easily be seen that the two belong to the same type. In the garden, when groups of 10–30 plants are compared, this similarity is of course far more striking. It is at once clear that *O. blandina* must be a true and pure *velutina*.

If we now try to resume this description in such terms as to distinguish a number of probable units, the combination of which might constitute the type of *velutina*, I might propose the following: (1) slender stature; (2) long internodes of the flower spike; (3) leaves narrow, folded longitudinally and smooth, that is, without bubbles; (4) flowers cup-shaped; (5) hairiness of all organs; (6) abundance of red color in the younger parts. It is obvious, however, that some of these points may go together and depend upon one unit, but on the other hand it must be conceded that this list may be far from complete. Most of these assumed units are recessive to the corresponding qualities of *O. Lamarckiana*, but the smoothness is dominant over the bubbles, which are evidently due to a lack of growth parallel to the surface of the blade of the leaf. This enables us to separate smoothness from the larger part

of the other characters by means of crosses, as we shall see when dealing with the hybrids.

I shall now describe the origin of *O. blandina* and the pedigree of the race derived from it. As a matter of fact, this mutant did not arise directly from *O. Lamarckiana*, but through another mutant race as an intermediate. This was the fertile race of *O. lata* issued from an original fecundation of my normal *O. lata* by means of the pollen of *O. semilata*. This race is described in my book *Gruppenweise Artbildung* on pp. 256, 257. In the fourth generation of this race, issued from the seeds of 1904 sown in 1905, 1907, and 1908, three specimens of *O. blandina* were observed, two in 1907 and one in 1908. Besides these, the cultures consisted of specimens of *O. lata* and *O. Lamarckiana* in the usual proportions, and of other mutants such as *O. nanella*, *O. oblonga*, and *O. scintillans*. The size of the cultures, however, was too small to calculate percentage figures.

The full name of the new type, therefore, would be *O. Lamarckiana* (mut. 1888 *lata*×mut. 1895 *semilata*) mut. 1907–8 *blandina*, leaving out the fecundation of the *lata* specimens of the pedigree by the pollen of *O. Lamarckiana*.

The second generation was derived separately from one mutant of 1907 and from that of 1908, both after guarded and artificial pollination. The seeds of the first mutant were sown in 1913 and gave a culture of 25 flowering individuals and 45 others which were pulled up shortly before flowering. Both groups were uniform, apart from the variation in the green color already mentioned. They were fully like the simultaneous culture of the third generation of the other strain, and for this reason this first line has not been continued.

The seeds of the mutant of 1908 were sown in 1912 and gave a culture of 67 flowering plants, all of which repeated the type of their parent. Besides these, I sowed part of the seed somewhat later in the season (June), and obtained a large group of rosettes of radical leaves, of which, however, only one survived our long, wet winter. This specimen flowered in 1913 on the main spike and on a number of branches, was very vigorous, but not strikingly stouter than the annuals of that summer.

In 1912 I saved the seeds of 4 self-fertilized plants separately. Two of them had been green from their first youth and the two others had been of a pale color in the beginning. The result was, in 1913, a small but distinct advantage of the two first sowings over the two latter sowings. I have chosen the first ones for the crosses to be described later on, and repeated this third generation in 1914 in order to give the plants more space and a better manured soil, and to compare such vigorous individuals with others growing in a dry and poor soil. The results of this comparison have been described elsewhere; they showed that the seeds produced by the two groups were different. Almost all the seeds of the strong plants contained a healthy germ, but among the seeds of the weaker individuals there were about 25 per cent of empty ones. The germination showed even a larger difference, giving about 80–90 per cent of seedlings for the normal seeds and only about 50 per cent for those of the weaker plants.[3] I shall return to this phenomenon in the second part of this article.

The fifth generation was derived, in 1915, from seeds of 4 self-fertilized individuals of 1914, chosen as the best ones among the stronger of the two groups. Sixty plants from one parent were planted in my experimental garden on good soil and with plenty of space, in order to be used for crosses. The remainder embraced about 3000 plants (between 600 and 800 from each parent) and were set out in another garden in order that the degree of mutability of this race might be studied. It was found to be rather small, although almost all of these plants have flowered and have been carefully tried at different stages of their evolution, from their germination to the time when their last flowers faded away in August and their first fruits ripened.

The result of these repeated inspections has been that 4 mutants, belonging to one type, were discovered, but that no other deviation could be observed. This gives a percentage figure of 0.1. If we compare this with the table given on p. 337 of my *Gruppenweise Artbildung*, we see that *O. blandina* falls into the group of those mutants (*O. nanella* and *O. rubrinervis*), the mutability of which has

[3] Über künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. **35**:175. 1915. In this article *O. blandina* has been provisionally designated as *O. Lamarckiana* mut. nov. B.

become much smaller than that of the parent species, which latter is given there as 2.2 per cent. The transition of one or more unit characters into the inactive condition was considered there as the probable cause of this change, and the same conception may obviously be applied to our present case.

Although the mutability of *O. blandina* is thus seen to be very small, it does not follow that it is wholly absent for other mutations than the one mentioned. Casual mutations parallel to those of *O. Lamarckiana* may be expected to appear from time to time, either in the pure strains or after hybridizations with other, still less mutable, species. This has occurred once in my garden. Among the offspring of a cross between *O. blandina* and *O. Cockerelli*, a species from Colorado, an individual arose in 1915 which showed the marks of *O. lata* combined with those of *O. Cockerelli*, and agreed with the description given in my book (p. 254). It proves that the mutability of *O. blandina* into *lata* is not wholly absent.

The mutation from *O. blandina*, 4 specimens of which occurred in my culture of 1915, was a strikingly new type, quite different from all the mutations produced by *O. Lamarckiana* and its derivatives until now. It was distinguished at once by its linear leaves, which could be seen in the boxes before the young plants were planted out on the beds. The 4 mutants were brought into the glass-covered part of my garden, where 2 of them have flowered. The 2 others remained small, produced stems, but died in the fall before making any flower buds. Of the flowering specimens one was also small and therefore was not used as a seed-bearer, but the other reached about 1 m. in height, was very richly branched, and bore, from July to October, many hundreds of flowers and fruits. All these flowers had the same type, consisting of narrow petals instead of the large cordate ones of the parental form. The petals did not belong to the type called *cruciata*, inasmuch as they had not the least sign of the sepalody characteristic of *O. cruciata* and its allies. Their color was uniformly yellow, not differing from that of *O. Lamarckiana*. The breadth varied from 0.5 to 1.5 cm. for a length of 3 cm. The form was ovate, with some small indentations along the margin, and the tip was narrowed and more or less spirally twisted. This latter mark, which was best visible during

the time of the most abundant flowering, has induced me to choose for this mutation the name of *O. spiralis*.

As to the other marks, they were probably all evolved under the influence of the very narrow leaves, which could not produce food enough for very stout individuals or organs. The leaves measured 5–6 mm. in breadth by a length of 8–10 cm.; they were smooth (without bubbles) as in the parent, not folded longitudinally, only a little hairy, and dark green. The internodes were long, reaching 2 cm. or more, and the foliage was therefore thin and the whole habit slender. The flower buds were less hairy than in *O. blandina*, but more so than in *O. Lamarckiana*, and broader than would be expected from the narrow petals. The stigma was above the anthers, which contained a good supply of pollen, making artificial self-pollination and crossing quite easy. The fruits were rather thin, and somewhat smaller than those of *O. blandina*, a little more conical, and less hairy.

It should be pointed out that the origination in 4 specimens, one from one parent and the 3 others together from another parent, is analogous to the production of *O. blandina* itself, which arose in 3 specimens from one lot of seeds. It points to an internal condition of heritable mutability and suggests the expectation that under a better climate and with more suitable conditions of cultivation the number of simultaneous mutations in the same direction might increase sensibly.

CROSSES OF O. BLANDINA WITH OTHER SPECIES.—In order to give proof that *O. blandina* is really the mutant *velutina*, I made some crosses with such species as are known to split *O. Lamarckiana* and some of its other derivatives into the twin hybrids *laeta* and *velutina*. It is obvious that with the loss of the active qualities of *laeta* this capacity of undergoing a splitting must disappear. The mutant *velutina* corresponds to the latent or inactive condition of these qualities, and if combined with splitting species it must therefore give rise only to one of the twins, the hybrid *velutina*. In other words, the crosses with these species must be expected to produce no *laeta* but only *velutina* hybrids, and these must be exactly the same as the *velutina* issued from the corresponding crosses of *O. Lamarckiana*.

Under normal conditions the splitting of this latter species occurs in nearly equal groups of both the twins, but as a matter of fact the external circumstances are, with us, often such as to diminish the percentage of *laeta* quite sensibly. These deviations, however, have been amply studied in my work on *Gruppenweise Artbildung;* they show what size the cultures must be in order to prove the absence of *laeta*. I cultivated 60–80 offspring for each culture, and repeated the same cultures during 2 years, making on the average 140 specimens for each cross. The first year I compared them with the hybrids *laeta* and *velutina* derived from the corresponding crosses of my new dimorphic mutant *O. cana*,[4] which happened to be at hand in a complete set; but the second year I have sown the twin hybrids issued from crosses of *O. Lamarckiana* itself with the same species as were used for the crosses of *O. blandina*. The material is externally the same in both cases, and quite as good for the comparison, but in the latter instance the proof is a more direct one. I grew the hybrids of *O. blandina* and the control cultures side by side, and compared them from the beginning of germination in February until the ripening of the first fruits in September. The differences between the two twins of a cross are large and striking,[5] from their very first leaves, and it was therefore impossible that a *laeta* among the hybrids of *O. blandina* could have escaped observation. As a matter of fact, no single *laeta* appeared, although altogether over 500 hybrids were cultivated.

For these crosses I chose 2 heterogamic and 2 isogamic species, and made the combinations in both groups in the opposite directions, so as to use *O. blandina* twice as a pistil and twice as a pollen parent. I made these pollinations in 1913, in the third generation of my race, choosing the most vigorous individuals which had had a normal green color from their very youth. The crosses with the heterogamic species were *O. syrticola* Bartlett[6]×*O. blandina* and *O. blandina*×*O. biennis Chicago*. Those with the isogamic forms

[4] De Vries, Hugo, New dimorphic mutants of the Oenotheras. Bot. Gaz. 62:249–280. *figs. 5.* 1916.

[5] For descriptions and photographs as well as for colored plates of the twins, see *Gruppenweise Artbildung.*

[6] This is the *O. muricata* L. of my *Gruppenweise Artbildung.*

were *O. Hookeri*×*O. blandina* and *O. blandina*×*O. Cockerelli*.[7] These combinations would have given the twins *laeta* and *velutina* if *O. Lamarckiana* had been used instead of *O. blandina*. Now, however, they gave only the hybrid *velutina*, showing that the splitting capacity of the parent species is absent in the mutant.

From these facts it follows that in *O. blandina* the *laeta* qualities of *O. Lamarckiana* are not only externally, but also internally, in the *velutina* condition. The former, therefore, is to be considered as a pure mutant *velutina*.[8]

I shall now describe the single crosses as briefly as possible.

O. syrticola×*O. blandina* was made on 2 biennial plants of the first named species in July 1913, the pollen of a green *blandina* being used in the one case, and that of a pale green one in the other case. The seeds of the first one were sown in 1914, those of the other in 1915. Both of them gave cultures of 70 healthy offspring, making a total of 140, of which 25 and 10 specimens were allowed to flower and to ripen their fruits, after the others had been pulled up, in June, before flowering. They were compared in every month of 1914 with the twins of *O. syrticola*×*O. cana*, and in 1915 with those from a cross of *O. syrticola*×*O. Lamarckiana*, made in 1913 on a specimen of the same group of biennial plants as used for the cross with *O. blandina*. All of the 140 hybrids were evidently *velutina* and exactly like those of the control cultures.

O. blandina×*O. biennis Chicago*.—This cross was made on two specimens of 1913, the pollen of the same parent plant being used in both of them. They gave uniform cultures of 60 and 80 plants, of which 25 and 10 were allowed to make long spikes of flowers and fruits. The others were annual plants also, but were thrown away in June, as soon as they reached a height of 30–40 cm., and showed their marks so as not to leave the least doubt concerning their *velutina* qualities. In 1914 I compared them with the hybrids of *O. cana*×*O. biennis Chicago*, and in 1915 with those of *O. Lamarckiana*×*O. biennis Chicago*, which I cultivated in the first generation

[7] For description and figures of these species see *Gruppenweise Artbildung*.

[8] Of course, other combinations, or combinations of the loss of the splitting capacity with other external marks must be admitted to be possible. As a matter of fact, such combinations occur from time to time, as, for example, in *O. oblonga;* see *Gruppenweise Artbildung*, p. 266.

as well as in the second, from another cross. The flowering individuals of all these crosses reached, in September, a height of more than 2 m., and all the *blandina* hybrids were, during all the stages of their evolution, like the *velutina* of the controlling cross.

O. blandina×*O. Cockerelli*.—Seeds of only one cross of 1913 were tried, both parents being annuals. One part was sown in 1914, another in 1915; size of the cultures, 60+80=140 specimens, of which 25 and 21 flowered. One of the latter was the *lata* mutant previously mentioned. In June 1914, there was not the least doubt concerning the identity of all the 60 specimens with *O.* (*cana*× *Cockerelli*) *velutina*, but in 1915 those young plants which had not been planted out in order to flower were a little too crowded. I therefore pulled out those which were indubitably *velutina*, and planted all the dubious ones on a separate bed, giving them just as much space as in ordinary cultures. About one-half of these (11 plants) flowered in September, but all of them displayed, at that time, the characters of *O.* (*Lamarckiana*×*Cockerelli*) *velutina*, so as to leave no room for doubt. No *laeta* has appeared among the offspring of this cross.

O. Hookeri×*O. blandina*.—Only one *Hookeri* was crossed in 1913 with one specimen of *blandina* of my race. The seeds gave 60 offspring in 1914 and 85 in 1915, of which 25 and 10 flowered. In the crosses of *O. Lamarckiana* and its derivatives with this Californian species, the *velutina* have almost the features of *O. Hookeri* itself, showing only a small influence of the other parent. I compared my hybrids in both years with first generation hybrids, using for the comparison both the reciprocal crosses of *O. cana* of 1913 in one year and the hybrids of a cross *O. Hookeri*×*Lamarckiana* of 1909 in the next year. Although the specimens, which were not allowed to flower, made stems of only a few centimeters, or stayed in the conditions of rosettes of radical leaves, the type of *O. Hookeri* was strongly pronounced in them. All of them had the long narrow leaves of *velutina*, and no *laeta* occurred in the whole culture of 145 plants.

Resuming these details, we see that 565 hybrids of *O. blandina* with splitting species have been studied, and that all of them bore the unquestionable features of the *velutina* of the corresponding crosses of *O. Lamarckiana* and of *O. cana*.

CROSSES OF O. BLANDINA WITH O. LAMARCKIANA.—If the *laeta* qualities have become latent and inactive in *O. blandina*, we should expect that this mutant would have acquired the property of splitting itself these qualities in *O. Lamarckiana*. The confirmation of this expectation must obviously strengthen the conclusion from which it started. And since *O. nanella* and other mutants may give rise to the same hybrid twins as the parent species, we may expect *O. blandina* to split them also. I made both the reciprocal crosses with the parent species and one with the dwarf, and all 3 cases have corroborated my conception. I made the crosses in 1913 in the third generation of my race, and cultivated the first generation in 1914, repeating it in 1915. The splitting occurred in all 3 cases as expected, giving nearly equal groups of the two types. One of these types exactly corresponded to *O. blandina* itself; in comparing it from its first youth up to the time of flowering and fruiting, I could not discover any difference. This one should be considered as the *velutina*, therefore, and will be called *O.* (*Lamarckiana*×*blandina*) *velutina* a.s.o. The other type was evidently a *laeta*. During some stages of its evolution it was almost wholly like *O. Lamarckiana*, but later it changed its appearance and displayed some of the characters which usually distinguish the different forms of the hybrid *laeta* from their parents. For this reason I shall call it *O.* (*Lamarckiana*×*blandina*) *laeta*, or briefly *O. blandina laeta*, implying by this name only the presence of one or more characteristics of the *laeta* type, but not necessarily all of them.

According to the species which determine the splitting in *O. Lamarckiana*, the types of hybrid *laeta* may be divided into two groups. One of them is small-flowered and ordinarily tall, corresponding to the *rigida* type described and figured in my book (pp. 73, 80, 81). To this type appertain the *laeta* produced by *O. biennis*, *O. muricata*, *O. Cockerelli*, and *O. biennis Chicago*. The other type has large flowers; it embraces only the *O.* (*Hookeri*× *Lamarckiana*) *laeta* and its reciprocal. The flowers have the same size in both parents, and therefore this size is not changed in the hybrids. In the cross of *O. Lamarckiana* and *O. blandina* the same rule prevails, the flowers of the hybrid being not rarely even somewhat stouter than those of the parents.

O. blandina laeta shows the greatest affinity to *O. Hookeri laeta*, not only in the flowers, but also in other respects, as, for example, in the stature at the time of flowering, which in both hybrids comes much nearer to that of *O. Lamarckiana* than any of the small-flowered hybrid *laeta*. Far more interesting, however, is the similarity in its behavior in the second generation, after *self-fertilization*. The *Hookeri laeta* are the only *laeta* as yet known to split; all *laeta* of other extraction and all the *velutina* as yet studied give a uniform progeny. But the *Hookeri laeta* split in every generation into *laeta* and *velutina* which are exactly like the original twins.[9] The same phenomenon is seen in *O. blandina laeta*, although as yet I have only cultivated one second generation from one cross. This was *O. blandina*×*O. Lamarckiana*, made in 1913. The first generation in 1914 gave 59 per cent *velutina* and 41 per cent *laeta*, and the progeny of the latter split into the same two types in 1915, giving 67 per cent *velutina* and 33 per cent *laeta*. Why the large-flowered *laeta* should split, but the small-flowered type remain constant, is a question which will have to be studied later.

O. blandina laeta has been, throughout its whole evolution, exactly the same type in the 3 crosses already mentioned, and whose progeny I cultivated in both years side by side. In the seed pans and the transplanting boxes the young plants are almost exactly like *O. Lamarckiana*, resembling this form far more than any of the hybrid *laeta* do. This condition prevails until the beginning of flowering, during which period the leaves of the stem are somewhat broader and less covered with bubbles than in the parent species. This difference is then seen to increase gradually and becomes evident in the lower bracts of the inflorescence, which are broad, especially at their base, smooth, and wholly or almost without bubbles. As the spike develops, the difference from the parental type becomes greater. The fruits are less crowded and somewhat stouter, and the plants gradually reach a greater height than specimens of *O. Lamarckiana* planted at the same time and under the same conditions. Although the differences are still small, apart from the smoothness of the leaves, the plants of *O. blandina laeta* cannot be mistaken for *Lamarckiana* during all the time of flowering, which may last more than 2 months.

[9] See the pedigrees in *Gruppenweise Artbildung*, p. 131.

It seems probable that the increased breadth and the diminished bubbles of the higher leaves of the stem and of the bracts of the inflorescence are expressions of a single change, which must consist in a thorough stretching of the blade parallel to its surface. If this be so, we may conclude that the bubbles, which are so characteristic of *O. Lamarckiana*, are due to some deficiency in this stretching and thereby constitute a recessive character. If this conclusion be granted, the smoothness of the leaves of *O. blandina* must be dominant in its crosses with *O. Lamarckiana*, and in this way be transferred to both of its twins, causing the one to be a *laeta* instead of a pure *Lamarckiana*. We are thereby provided with a beginning of an experimental analysis of the marks of mut. *velutina*, as already discussed.

Here I might insert some considerations concerning the mutative origin of *O. blandina*. We have seen that *O. Lamarckiana* and *O. nanella*, when crossed with this new form, repeat its characters in part of the offspring. In the same way a mutant *velutina* may be produced by the conjugation of a mutated sexual cell with a normal one. Thus it is not necessary to assume the accidental meeting of two mutated gametes, which would obviously make the chance of the mutation occurring very much smaller still. It is sufficient to suppose that only the female elements of the original *O. laeta* have mutated in this way, although we cannot know whether this change might not have taken place in the male cells. And since *O. blandina* behaves as an isogamous species, both hypotheses seem to be equally probable. In both cases mutants of the *laeta* type should be expected to appear also, but as they would be very rare and not discernible in the beginning from the *Lamarckiana* specimens which always develop out of a part of the seeds of *O. lata*, they would surely have been overlooked in the years 1907–1908, when the mutation into *blandina* occurred.

I shall now give a more detailed description of my experiments. The crosses were made in 1913 and the first generation was cultivated twice for every cross, once in 1914 and once in 1915.

O. blandina×*O. Lamarckiana*.—A biennial specimen of the latter form was chosen and its pollen placed on the stigma of two individuals of the thoroughly green type of *O. blandina*. The seeds of one cross were sown in 1914, and those of the other in 1915.

The first culture consisted of 23 *laeta* and 34 *velutina*, making a total of 58, with 41 per cent *laeta*. In 1915 the figures were 46 *laeta* and 39 *velutina*, or 54 per cent *laeta* in 85 specimens. Although the size of the cultures was small, they evidently point to a division in nearly equal groups. The two types were clearly different from the beginning and could easily be counted out in June before the production of the stems. In 1915 I separated them in March, at the time of planting into the boxes, in order to control my estimate later on, and in April planted the *laeta* in one group and the *velutina* in another half of the bed. In 1914 I had 25 and the following year 10 flowering plants, half of which belonged in each case to the *laeta* type and the other half to the *velutina* type. The *laeta* have already been described; the *velutina* were in no respect and at no time different from ordinary *O. blandina*.

The second generation from seed of one of the *velutina* plants embraced 30 flowering and 40 younger specimens, all of which exactly repeated the marks of their parent. From the seeds of one self-fertilized *laeta*, however, I got the splitting group already described. Its two types were the same as in the previous generation. I recognized the splitting in the seed pan, but counted them only in June after planting out 15 *laeta* and 15 *velutina*. All in all I had 80 plants, of which 26 were *laeta* and 54 *velutina*, or 33 per cent *laeta*, which is somewhat less than in the first generation. All the 30 specimens of the bed richly flowered and ripened their first fruits before being thrown away.

O. Lamarckiana×*O. blandina*.—A biennial plant of the species was crossed in 1913 with a green individual of the mutant. The seeds were sown partly in 1914 and partly in 1915. They gave the same two types as in the reciprocal cross. During the whole lifetime there were no visible differences. In the first year I had 60 plants with 22 per cent *laeta*, and in the second year 108 specimens with 25 per cent *laeta;* the remainder were *velutina*. Of these, 25 and 10 flowered, in about equal groups for both types, having been recognized and sorted out at the time of planting. The other plants were cultivated till the end of June.

O. blandina×*O. nanella*.—Two green individuals were fertilized in 1913 by the pollen of my race of *O. Lamarckiana* mut.

nanella; the seeds of the one were sown in 1914 and of the other in 1915. There were no dwarfs in this first generation, but only *laeta* and *velutina*, which were just like those of the crosses already described. I had 90 and 72 plants, with 74 and 67 per cent *laeta*. There were 25 and 10 flowering plants belonging equally to the two groups; the others were large rosettes in June.

If we compare the percentages of *laeta* given with one another we find for *O. blandina*×*O. Lamarckiana* 41 and 54 per cent, for the reciprocal cross 22 and 25 per cent, and for the experiment with the dwarfs 74 and 67 per cent; finally, for the second generation of the first cross 33 per cent. The average of all these figures is 45 per cent *laeta*, which comes as near to equality of the two groups as may be expected. The deviations from this mean are probably due mainly to the choice of the parents and to their cultural conditions.

O. rubrinervis×*O. blandina*.—Besides the 3 crosses already mentioned and discussed, I have also made the two reciprocal crosses with my race of *O. rubrinervis*. In the first generation they split in the same way, the only difference being that instead of the *laeta* another type arises. This is the *subrobusta*, which appears in the hybrid splittings of *O. rubrinervis* with other derivatives of *O. Lamarckiana*, as described in my *Gruppenweise Artbildung*. No differences were observed, although the comparison lasted from germination till the ripening of the fruits. The other type was the same as in the crosses already dealt with, and exactly like the parental type of *O. blandina*.

The cross was made in 1913 between an individual of my pure race of *O. rubrinervis* and a specimen of the third generation of *O. blandina*. One part of the seeds was sown in 1914 and another in 1915. In the first year I had 60 plants with 32 per cent *blandina*, and cultivated 18 *laeta* and 7 *blandina* until the ripening of their fruits. In the last named year I had 77 specimens, of which 61 per cent were *blandina* and of which 5 *laeta* and 5 *velutina* were left to flower. All in all, the cultures embraced 137 plants, with 45 per cent *blandina*. The others were all *subrobusta* and not different from the *subrobusta* cultures of those years resulting from other crosses.

O. blandina×*O. rubrinervis*.—For this cross I used two specimens of *O. blandina* of the third generation in 1913, the one being a pale green and the other a normal color. In 1914 each of the cultures embraced 60 plants, of which 25 flowered. The percentages for *blandina* were 48 for the green, but only 20 for the pale parent. For this reason I repeated the latter culture in 1915 and obtained from 70 plants 47 per cent *blandina*. The types of *subrobusta* and *velutina* in these cultures were exactly the same as those from the reciprocal cross.

The percentages given are obviously of the same type as those for the splitting into *laeta* and *velutina* and come as near to equality for the two types as may be expected under ordinary conditions of cultivation. I propose to grow the second generation next summer.

THE VIABILITY OF THE SEEDS OF O. LAMARCKIANA MUT. VELUTINA.—Besides the external differences between our new mutant and the parent species, there is another mark which lends a high interest to the new form. This is found in the seeds. The seeds of *O. Lamarckiana* differ from those of almost all other species (with the exception of *O. suaveolens*) in containing a large proportion of empty grains, even under the most favorable conditions of life. More than one-half of the seeds have no germ at all, although externally they are, as a rule, not distinguished from the normal ones. RENNER[10] has studied the development of these empty seeds and found that their germ is fecundated and undergoes one or two cell divisions, but then stops and dies off. He considers this phenomenon as a hereditary character of the species. It runs parallel, in this respect, to the rudimentary ovules described by GEERTS, which are characteristic of the whole group of the Oenotheras. Besides this type of empty seeds a less or larger number usually occur which stop their development at a much later stage. The proportion of these can be diminished by a better culture, and therefore they may be considered as a result of the crowding of the seeds in the capsules, combined with the limited amount of nourishment available for them.

Our new mutant *velutina* produces hardly any abortive seeds, at least under normal conditions of culture. I tried the seeds from

[10] RENNER, O., Befruchtung und Embryobildung bei *Oenothera Lamarckiana*. Flora 7:115–150. 1914.

purely pollinated capsules of 4 specimens of my culture of 1915, which was the fourth generation of my race. I took them carefully out of the fruits, mixed those of 5 successive capsules of the same spike, and counted 200 grains from each lot. I soaked them in water, pushed this into their seed coats by means of a pressure of 8 atmospheres for about 24 hours, and afterward laid them out to germinate in small glass tubes in a stove at 30° C.[11] Within 6 days the larger part of the seeds germinated, giving percentages of 85, 84, 73, and 70 for the 4 lots. I then opened the remaining grains and found fully developed germs in almost all of them. The percentage of germs, being the sum of the two trials, came to 99, 96, 96, and 93, and there is no doubt that if they had been left in the stove for a longer time, almost all of the resting germs would have shown signs of germination.

The proportion of rapidly germinating grains and that of empty ones depend in a high measure upon the external conditions of life. During the summer of 1914 I cultivated 2 lots of individuals from seeds of the same parent plant, giving to one of them the ordinary favorable conditions of my garden, and keeping the others in a dry soil without manure. The seeds of the 2 most vigorous specimens of both lots, taken from self-fertilized capsules, were tried. They contained 99 and 99 per cent of germs for one group, but only 72.5 and 73.5 per cent for the other group. The same effect was shown in the amount of grains which germinated within the first 6 days. The figures were 80–88 per cent in one case and 53 per cent in the other case.[12]

The loss of the hereditary property of producing about 50 per cent of empty grains constitutes a latent mutation accompanying the visible changes in the external structure. The same loss accompanies the mutation into *gigas*, *rubrinervis*, and some newer mutants, but does not occur in such cases as those of *O. lata*, *O. scintillans*, *O. cana*, and others (see footnote 4).

[11] This method has been followed in all the experiments to be described in the text. It is described in the following papers: The coefficient of mutation in *Oenothera biennis* L. BOT. GAZ. **59**: 190–194. 1915; and in Über künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. **35**:161–176. 1915.

[12] Biol. Centralbl. **35**:174–175, where *O. blandina* was provisionally indicated as mut. nov. *B.*

I made a series of crosses in order to study the nature of this latent mutation of *O. blandina*. They led to the discovery of some points which seem to deserve a more thorough study than I could give them until now.

In the first place, it is to be expected that in crosses with those species which do not produce such empty seeds the high figures of both parents will simply be repeated. I tried these cases, determining the amount of normally developed germs in lots of 200 seeds each, after the method already described. I made the crosses in 1914 and 1915, and in most cases in both the reciprocal directions. The seeds were taken from 5 successive fruits, and carefully prepared so as not to lose any small grains. The results are given in table I.

TABLE I

Cross	Percentage of germs*		
	Cross	Cross	Reciprocal
O. biennis ×blandina.....	99	—	91
O. biennis Chicago× "	95	89	94
O. Cockerelli × "	99	95	87
O. Hookeri × "	94	91	—
O. syrticola × "	87	83	—

* The dash (—) means that the cross has not been tried.

In some instances I cultivated the first generation of the hybrids in 1915 and tried their seeds after self-fertilization. I got almost the same figures: *O. blandina*×*Chicago* 86 per cent, *O. Hookeri*× *blandina* 97 per cent, and *O. syrlicola*×*blandina* 93 per cent.

In trials with other species the hereditary property of *O. Lamarckiana* of making germs in only one-half of its seeds is recessive to the normal condition of producing almost only normal grains. It behaves in the same way in crosses with *O. blandina*. The results are given in table II.

Of course it is to be expected that those mutants which themselves give high percentages after self-fertilization will do the same in their crosses with the *velutina* mutant (table III).

The crosses were made in 1915. *O. rubrinervis* is the same strain as used in my *Gruppenweise Artbildung*. *O. erythrina* is a new mutant from *Lamarckiana*, of the type of the hybrid form *subrobusta* of that book; and *O. deserens* is a mutant from *O. rubri-*

nervis, originated through the loss of the typical red color, but without change in the brittleness of the stem. These new mutants will have to be dealt with in another article.

TABLE II

Cross	Culture	Percentage of germs	
		Cross	Reciprocal
O. blandina ×Lamarckiana....	1914	94	—
" × "	1915	97	95
" ×nanella.....	1915	99	—
O. Lamarckiana×blandina.....	1914	90	—

The most interesting question in this situation, however, is that concerning the seeds of the first generation of the crosses between *O. Lamarckiana* and *O. blandina*. I made the crosses in

TABLE III

Cross	Percentage of germs	
	Cross	Reciprocal
O. blandina×rubrinervis.....	91	97
" ×erythrina.....	99	—
" ×deserens.....	99	100

1914, cultivated the hybrids in 1915, and tried their self-fertilized seeds during the winter. From each of the 3 crosses I had 5 vigorous specimens of the *laeta* type, and an equal number of the *velutina* type. I shall deal with the *laeta* first (table IV).

TABLE IV

SELF-FERTILIZED SEEDS OF *laeta* SPECIMENS OF THE FIRST GENERATIONS

Cross	Percentage of germs				
O. Lamarckiana×blandina.....	98	98	97	96	95
O. blandina ×Lamarckiana.....	96	96	95	92	36
" ×nanella.....	96	96	43	41	39

The 3 crosses may be taken as instances of one type and combined on this ground. We see that a splitting occurs, 11 individuals having the high percentages of the one parent, and 4 others

having the low figures of *O. Lamarckiana*. This splitting may be considered, therefore, as amphiclinous,[13] 73 per cent of the hybrids belonging to the one and 27 per cent belonging to the other parental type. The splitting is analogous to that of the cross *O. Lamarckiana*×*nanella*, which gives, under ordinary circumstances, in the first generation about 78 per cent tall and 22 per cent dwarfish specimens. It must be pointed out, however, that here the germs, which belong to the next generation, are dealt with as a mark of the first generation. In this respect they may be compared with the rudimentary ovules of GEERTS, which are not fertilized.

I tried also the *blandina* plants of the same crosses, but had fertilized only two specimens of each, which is too few to study this phenomenon. I found 77 and 69 per cent, 67 and 60 per cent, and 71 and 70 per cent of good grains among their seeds. It is remarkable that all of these plants had about the same figure, which is far less than that of the specimens of the pure race of *O. blandina* (about 95 per cent), but a splitting did not show itself.

The same difference as between the *laeta* and extracted *velutina* was shown by the *subrobusta* and the *velutina* from the crosses with *O. rubrinervis*. I found the figures as given in table V.

TABLE V

Cross	Hybrid	Percentage of germs		
O. rubrinervis×blandina	subrobusta	97	96	—
O. blandina ×rubrinervis	"	96	96	96
O. rubrinervis×blandina	blandina	70	68	—
O. blandina ×rubrinervis	"	80	75	—

It is possible, however, that this difference is only an effect of the higher exigencies of the plants of the type of *blandina*, since we have already seen that their amount of good grains is easily diminished by an unfavorable culture. Moreover, this character is not a constant one, for in trying the *blandina* plants of the second generation I found their contents of normal embryos complete, no single empty grain being found in lots of 200 seeds of two such plants.

[13] Über amphikline Bastarde. Ber Deutsch. Bot. Gesells. 33:461–468. 1915.

DEVRIES on OENOTHERA

It is necessary, of course, to study the progeny of the *laeta* plants with a high and of those with a low percentage figure. Until now, however, I have had only an opportunity to study the offspring of one *laeta* of the first type, since I happened to have fertilized only that one in 1914. Its seeds contained 96 per cent of germs. I cultivated 15 specimens of *laeta* from these seeds, and found, for self-fertilized seeds of each of them, percentages between 91–97, with a mean of 95. In this instance, therefore, the high percentage seems to be constant.

The main result of this inquiry is that *O. Lamarckiana* mut. *velutina* has lost the property of the parent species of producing about one-half of empty grains, that this property is recessive to the normal production of almost only good grains, and that a splitting is observed in the first generation, which seems to follow the type of amphiclinous hybrids, as in the case of *O. Lamarckiana*× *O. nanella*. A further study is required to elucidate these points, especially the behavior of the seeds as a mark of the generation which produces them.

Summary

1. *O. Lamarckiana* mut. *velutina*=*O. blandina* arose from my family of *O. Lamarckiana* mut. *lata*×*semilata* among seeds of the third generation saved in 1904, in 3 specimens. Of one of these I cultivated a second generation and of one of the others 4 successive generations, embracing together over 3000 plants.

2. All these plants were exactly alike with the exception of 4 mutants which constituted a new type, *O. spiralis*. The mutation coefficient was 0.1 per cent, or about the same as for *O. rubrinervis* and *O. nanella*, and much smaller than that for *O. Lamarckiana*.

3. For the appearance of the original mutation only one sexual cell needs to be mutated, since in combining with a normal gamete it may give rise to *O. blandina*, as is shown by the splitting of both the reciprocal crosses of this form with *O. Lamarckiana*. The splitting goes into nearly equal groups of specimens like *O. blandina* and of *laeta*.

4. *O. Lamarckiana* mut. *velutina* resembles the hybrids of the type of *velutina* so much as to be considered one of them. Among

them it is the most like *O.* (*Lamarckiana*×*O. biennis Chicago*) *velutina*, without the marks of the second parental species, however. It is slender, with long internodes in the spike, and with flowers as large as those of *O. Lamarckiana*.

5. *O. Lamarckiana* mut. *velutina* is distinguished from its parent species in a very striking character. It has lost the property of producing about one-half of empty grains; almost all of its seeds contain healthy and well developed germs and germinate easily. This new quality is dominant over that of the parent. It is the same as in almost all the older species of the genus.

6. Moreover, *O.* mut. *velutina* is distinguished from *O. Lamarckiana* at least in one other dominant character, the smoothness of its leaves at the time of flowering. Secondly, it is distinguished in quite a number of characters, which seem to be more or less independent of one another, namely, slender stature, long internodes of the flower spike, narrow and longitudinally folded leaves and bracts, and cup-shaped flowers. Besides these, the richness in red color and the hairiness of all organs, especially in their youth, are very striking marks.

7. In crosses with those species which split *O. Lamarckiana* and some of its other derivatives into the twin hybrids *laeta* and *velutina*, the *O.* mut. *velutina* produces only hybrids of the *velutina* type.

8. In crosses with *O. Lamarckiana* and *O. nanella*, these forms are seen to be split by *O.* mut. *velutina* into twin hybrids, which correspond to the twins produced by other species with them, but which, of course, lack the characters of those other parents. The twins of *O. blandina* may be considered as pure *laeta* and pure *velutina*, therefore, the former having smooth leaves and bracts in the summer, the latter being identical with *O. blandina* itself.

9. The study of our new mutant reveals the existence of at least two recessive characters in *O. Lamarckiana*, namely, the bubbles of the leaf blade and the presence of typical empty seeds.

BOTANIC GARDEN
AMSTERDAM

EXPLANATION OF PLATE I

At the right, *Oenothera Lamarckiana* mut. *velutina* (*O. blandina*); at the left, *O. blandina* mut. *spiralis*.

INFLUENCE OF THE LEAF UPON ROOT FORMATION AND GEOTROPIC CURVATURE IN THE STEM OF BRYOPHYLLUM CALYCINUM AND THE POSSIBILITY OF A HORMONE THEORY OF THESE PROCESSES

JACQUES LOEB

(WITH THIRTY FIGURES)

In two former publications[1] it was shown that while the stem of *Bryophyllum calycinum* prevents or retards the development of roots and shoots in the notches of a leaf, conversely the leaf accelerates the development of roots and shoots in a stem; since in a stem deprived of all leaves the roots and shoots develop later and grow more slowly than if a leaf is left on the stem. The two phenomena found a common explanation in the assumption that the leaf furnishes substances to the stem which accelerate the organ formation in the latter, while if these substances are not "sucked away" from the leaf by the stem they will accelerate the growth of roots and shoots in the notches of the leaf. These substances may be water or solutes.

In these experiments it was noticed that the leaf has also an accelerating effect upon the geotropic curvature of the stem. When stems of *Bryophyllum* are suspended horizontally by 2 threads in a jar saturated with water vapor, they will bend, becoming convex on the lower, and concave on the upper side (fig. 1), and this bending continues until finally the stems assume the shape of a U. This geotropic bending is a slow process when the stem contains no leaf, but is considerably accelerated if a leaf is left on the stem (fig. 1). The position of the leaf has a great influence, not only on the velocity of the geotropic bending and the region of the stem in which it occurs, but also upon the formation of organs in the stem. The description of this influence and of the apparently close connection between the two groups of phenomena will form the subject of this paper.

[1] LOEB, J., BOT. GAZ. **60**:249–276. 1915; **62**:293–302. 1916.

Straight stems, at least 10 cm. long, were selected for these experiments. The growing region and the 2 apical nodes were removed, the pieces chosen for experimentation containing about 4–7 or 8 nodes. Each stem was suspended horizontally by 2 threads (fig. 1), one at each end, in a jar the bottom of which was filled with water. The jar was loosely covered with a glass plate so that the stems were surrounded by an atmosphere almost completely saturated with water vapor.

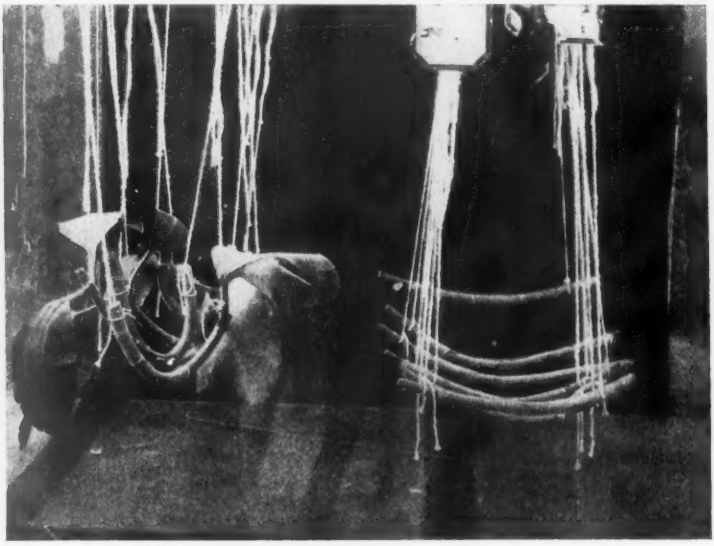

FIG. 1

The writer was rather surprised to find that stems deprived of their growing region should show geotropic curvatures. These curvatures were not confined to the nodes as is the case in grasses, but whole regions of the stem, both nodes and internodes, bent (fig. 1). We shall see later that the bending was accomplished by an increase in the length of the cortex of the under side of the horizontally placed stem, while the upper side of the stem was bent passively as a consequence of this growth.

I. Influence of the presence and absence of leaves

Fig. 1 illustrates the influence of the presence and absence of leaves upon the rapidity of geotropic curvature in the stems. The 6 stems to the right had no leaves and bent very slowly; the 6 stems to the left had each 2 leaves at the most apical node (while the other leaves were removed) and bent much more rapidly. The photograph was taken on the eleventh day after the experiment was begun. It is noticeable also that the stems containing leaves

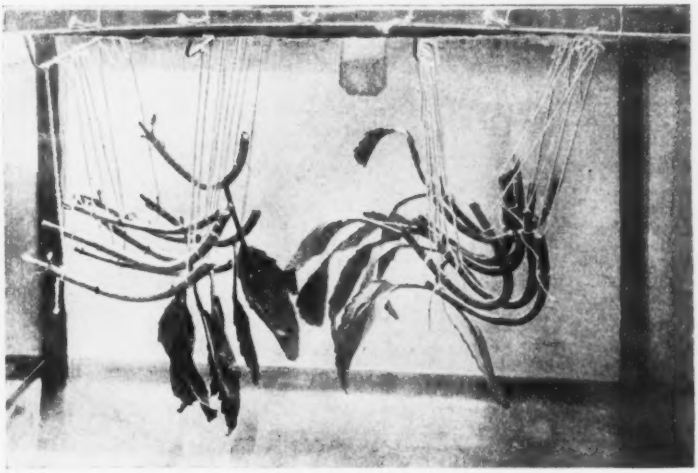

Fig. 2

formed roots (in their basal nodes) much more rapidly than the stems deprived of all leaves. This experiment, representing the accelerating influence of the apical leaves upon both root formation and geotropic curvature, never fails; and the same may be said of most of the experiments described in the following pages.

II. Influence of the position of the leaf on the stem upon geotropic curvature and organ formation

In the following experiment only one leaf was left on the stem. It was found that it made a great difference whether this leaf was at the apex or at the base. This is illustrated by fig. 2. On the

right hand in the photograph are 6 stems having one leaf left at the apex. These stems bent so rapidly and strongly that they soon reached a U-shape. On the left are 6 stems having a leaf left at the base. In this case the curvature is generally much less than

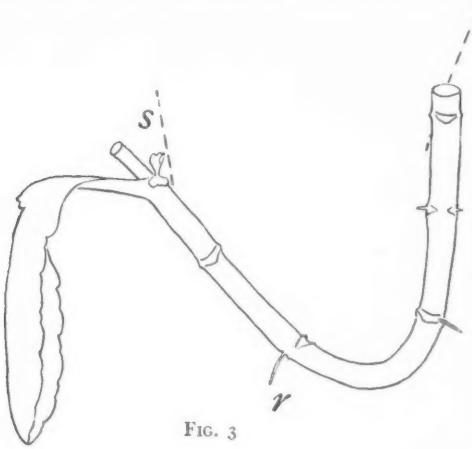

FIG. 3

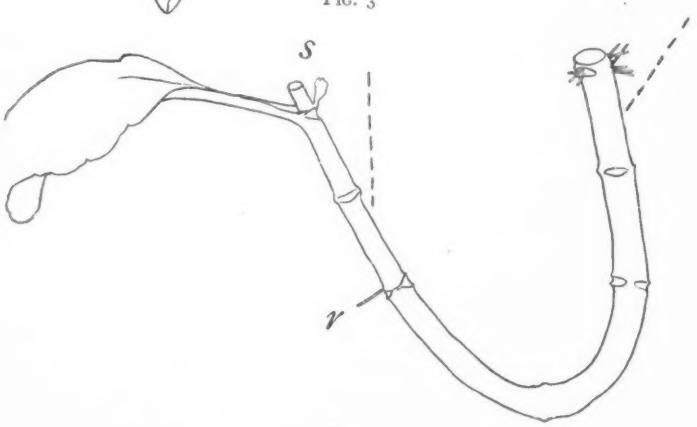

FIG. 4

when the leaf is at the apex. Both groups of stems had been suspended horizontally and both had been put into the jar at the same time. The photograph was taken on the eleventh day. This experiment also is always successful.

It is noticeable, incidentally, that while the leaf at the base accelerates the shoot formation, the one at the apex accelerates root formation. The more rapid geotropic curvature occurs in those stems in which the root formation is favored.

Aside from the influence of the position of the leaf upon the velocity and extent of the curvature, an equally striking influence exists between the position of the leaf and the localization of the curvature in the stem. When the leaf is at the apex, the curvature appears near the second node behind (basally from) the leaf (figs. 3, 4), and is confined chiefly to this region and possibly to the next node located more basally. The drawing was made 10 days after the experiment began.

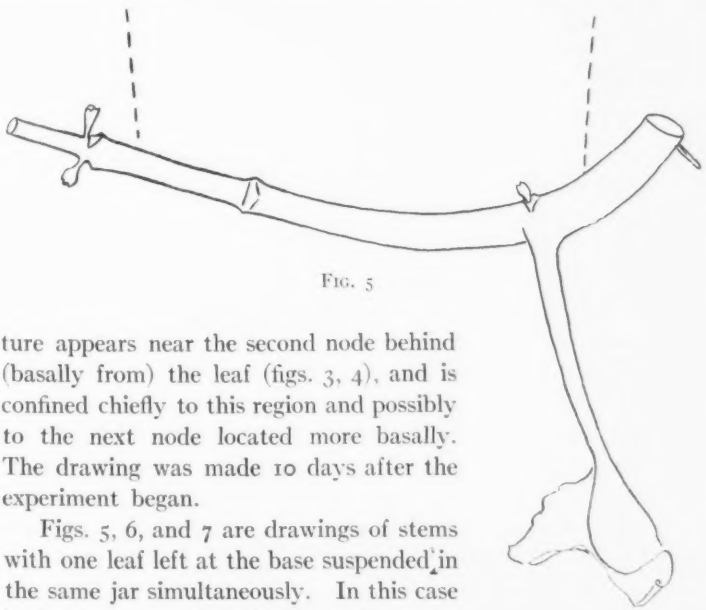

FIG. 5

Figs. 5, 6, and 7 are drawings of stems with one leaf left at the base suspended in the same jar simultaneously. In this case little curvature takes place and the curvature which occurs is near the region where the leaf is located. Fig. 6 is an extreme case. It seems that the amount of curvature increases with the length of the piece of internode left behind the basal leaf. The photograph in fig. 2 shows also the difference in the localization of curvature according to whether the leaf left is at the apex or at the base.

In the experiments thus far mentioned the leaf was on the lower side of the stem. When the leaf is left on the upper side of a

horizontally suspended stem, the geotropic bending is slower than when it is on the lower side, but the bending will also be much more rapid and more intense when the leaf is left at the apex (figs. 8, 9, 10) than when it is left at the base (figs. 11, 12, 13). In the latter case the bending is again slight, and what little curvature occurs is confined to the immediate neighborhood of the basal leaf. Fig. 13

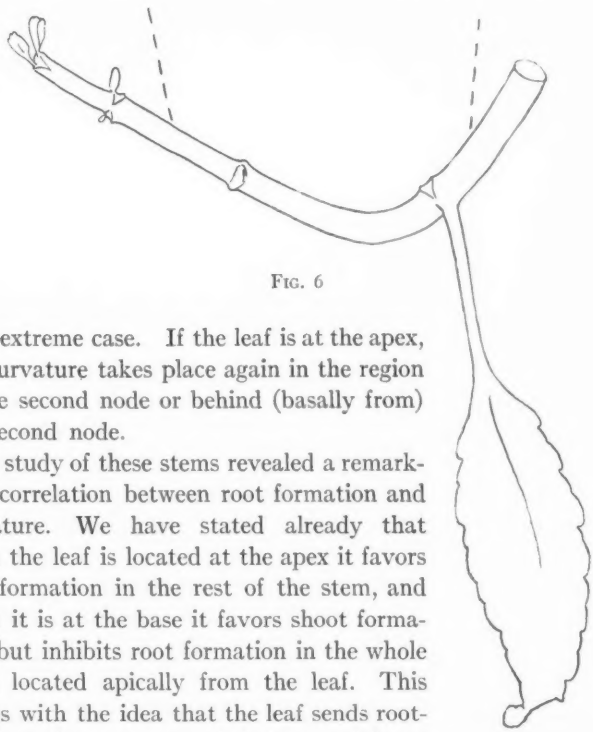

FIG. 6

is an extreme case. If the leaf is at the apex, the curvature takes place again in the region of the second node or behind (basally from) the second node.

A study of these stems revealed a remarkable correlation between root formation and curvature. We have stated already that when the leaf is located at the apex it favors root formation in the rest of the stem, and when it is at the base it favors shoot formation but inhibits root formation in the whole stem located apically from the leaf. This agrees with the idea that the leaf sends root-forming substances toward the base and shoot-forming substances toward the apex. We notice that the geotropic curvature is favored or accelerated most in those stems in which an apically located leaf is left, and in such stems root formation is favored also. The correlation between root formation and geotropic curvature is still more striking, however, if we consider the location of the roots formed. When the leaf left is at the apex,

roots usually appear in the second (and often the fourth) node behind the leaf, where the geotropic curvature also begins. This is obvious in *r*, figs. 3, 4, 8, 9, and 10. When the leaf left is at the base, the root formation is considerably less (as is also the geotropic curvature), and what there is of root formation occurs again in the region where the main geotropic curvature also occurs; namely, in the lower basal node (*r*, figs. 11, 12, 13). When the leaf is at the base, root formation is more favored when the leafless basal node

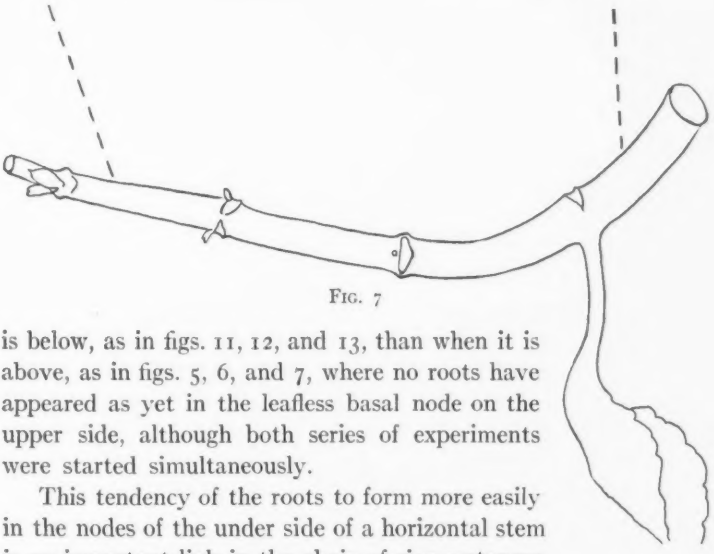

FIG. 7

is below, as in figs. 11, 12, and 13, than when it is above, as in figs. 5, 6, and 7, where no roots have appeared as yet in the leafless basal node on the upper side, although both series of experiments were started simultaneously.

This tendency of the roots to form more easily in the nodes of the under side of a horizontal stem is an important link in the chain of circumstances connecting root formation and geotropic curvature, since the growth causing this curvature is confined to the cortex of the under side of the stem.

III. EXPERIMENTS ON STEMS SPLIT LONGITUDINALLY

In order to find out the mechanism of geotropic curvature, stems were split lengthwise and suspended horizontally in jars saturated with water vapor. Each half stem had one leaf left either at the apex or at the base. Figs. 14–20 give the typical results of a series of such experiments on the seventeenth day.

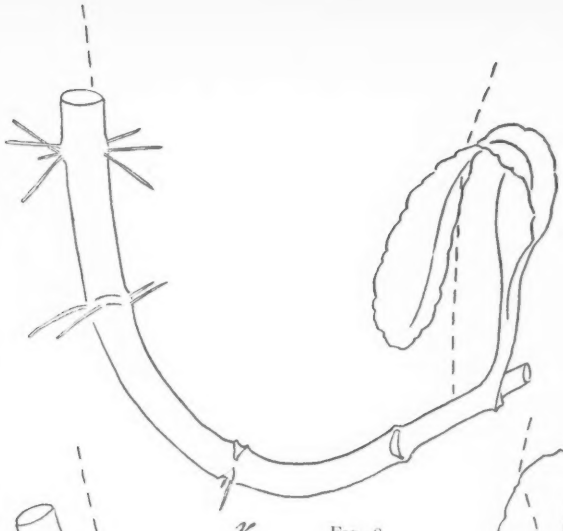

FIG. 8

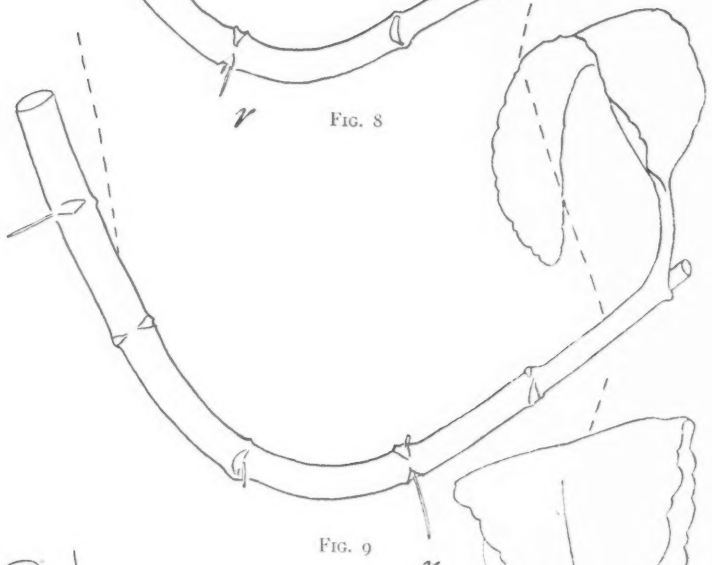

FIG. 9

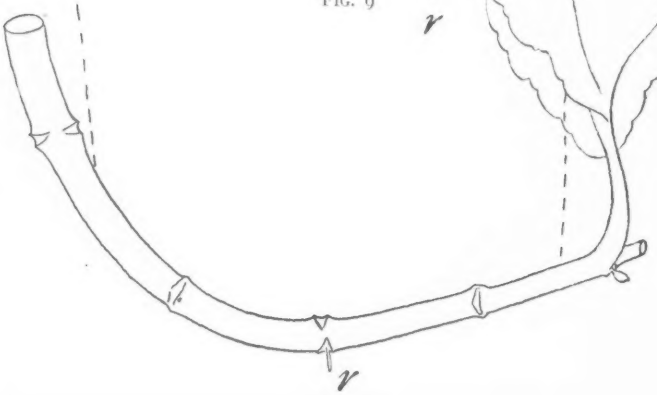

FIG. 10

Figs. 14, 15, 16, and 17 give the appearance of the lower halves of the stems (that is, stems suspended horizontally in such a way that

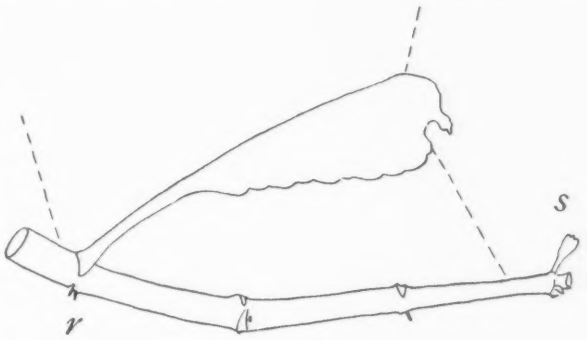

FIG. 11

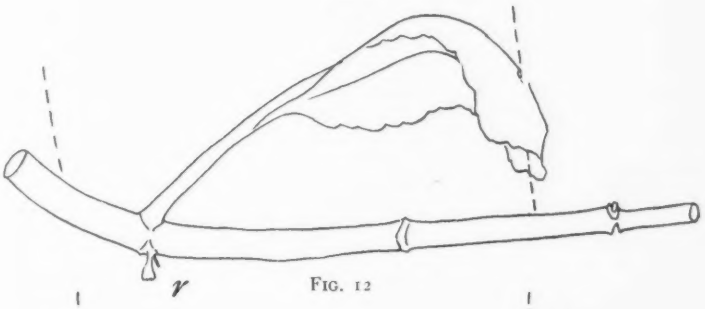

FIG. 12

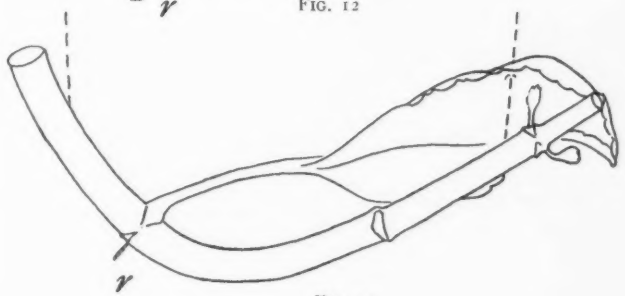

FIG. 13

the cortex was below, and the cut surface above) on the seventeenth day. When the apical leaf is preserved, the bending is rapid and extensive, as in fig. 17. When the basal leaf is preserved (figs. 15,

16), either no bending occurs, as in fig. 15, or it is confined to the region of the leaf or of the first node in front of the basal leaf (fig. 16). When no leaf is left, as in fig. 14, a slow bending takes

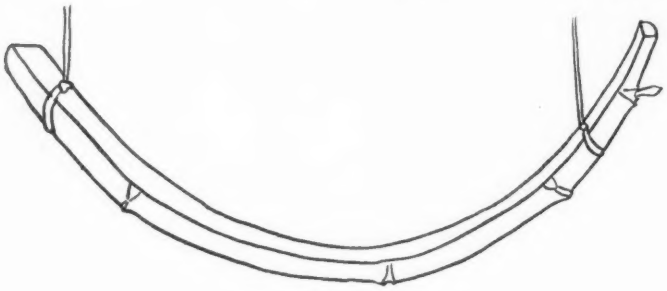

FIG. 14

place, more rapid and extensive than in fig. 15, but less rapid than when the apical leaf is left, as in fig. 17. In the other halves of the stem which were suspended with the cortex above (figs. 18, 19, 20), practically no geotropic bending takes place, for the reason that

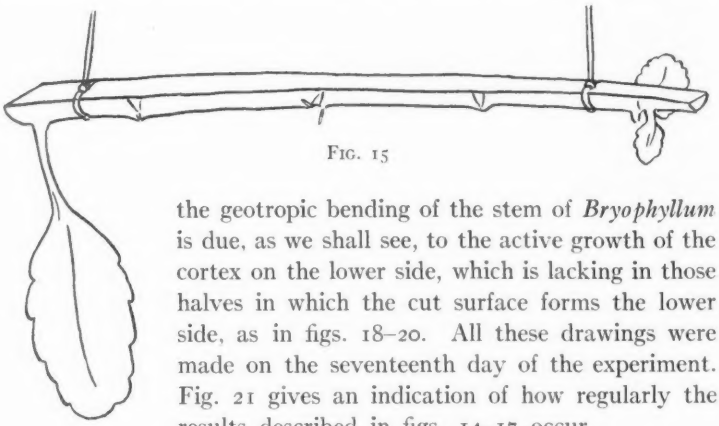

FIG. 15

the geotropic bending of the stem of *Bryophyllum* is due, as we shall see, to the active growth of the cortex on the lower side, which is lacking in those halves in which the cut surface forms the lower side, as in figs. 18–20. All these drawings were made on the seventeenth day of the experiment. Fig. 21 gives an indication of how regularly the results described in figs. 14–17 occur.

It was of interest to study the reaction of split stems in which the leaf was above and the cortex below. For this purpose it was necessary to split the stem only to the apical or basal node in which the leaf was preserved (figs. 22, 23), but not in its entire length.

When the leaf is at the apex and above (fig. 22), geotropic curvature of the stem occurs, but not so rapidly as when the leaf is below;

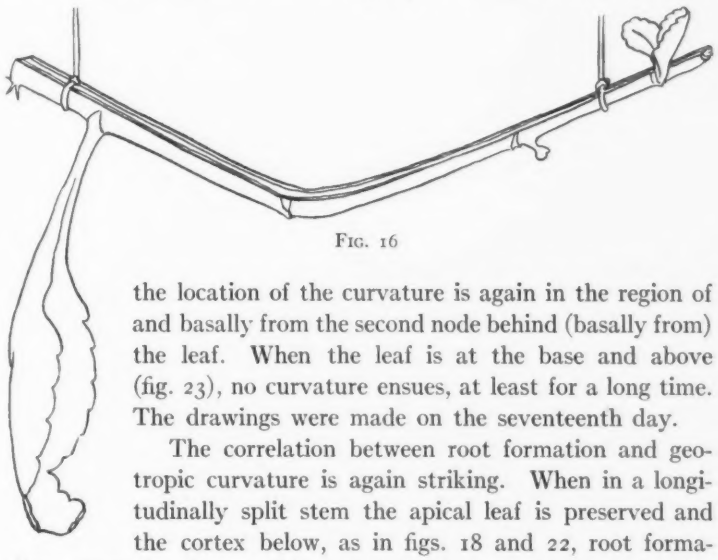

FIG. 16

the location of the curvature is again in the region of and basally from the second node behind (basally from) the leaf. When the leaf is at the base and above (fig. 23), no curvature ensues, at least for a long time. The drawings were made on the seventeenth day.

The correlation between root formation and geotropic curvature is again striking. When in a longitudinally split stem the apical leaf is preserved and the cortex below, as in figs. 18 and 22, root formation occurs in the second node behind the leaf, in the region

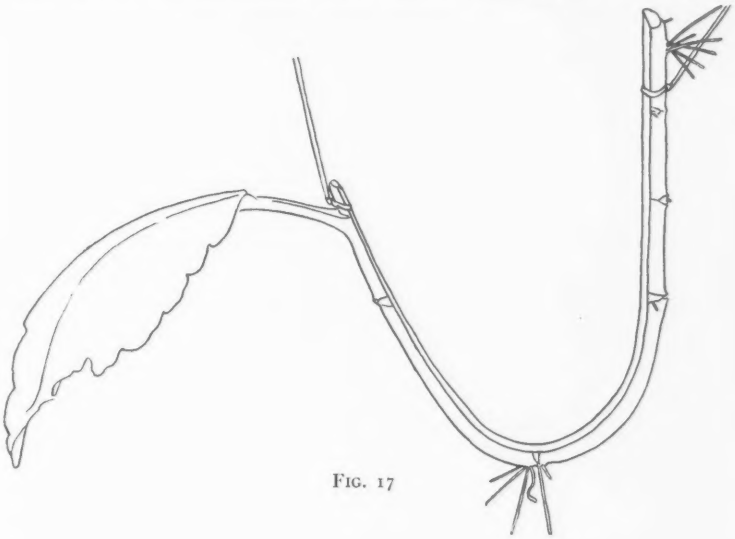

FIG. 17

where the curvature also occurs. Roots appear also at the basal nodes and sometimes at the cut basal surface of these

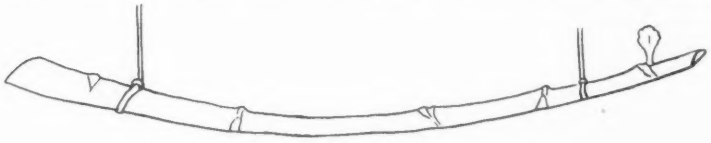

FIG. 18

stems. When the basal leaf is preserved and this leaf is below (fig. 15), no root formation takes place generally, or not for a long

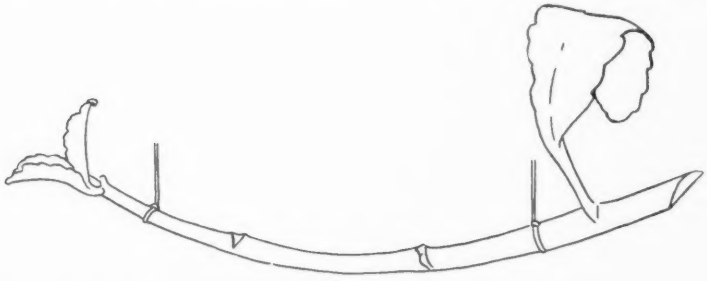

FIG. 19

time at least; while when the leaf is above (fig. 23), such root formation takes place in the basal node on the under side of the stem opposite the leaf. When the cortex is above and the leaf at the apex, root formation will occur, but chiefly or most rapidly in the basal node, and later in the next or the 2 nodes next to them (fig. 20). When the leaf is at the base or when the stem has no leaf, no root formation occurs in the cases where the cortex is above, at least for a long time.

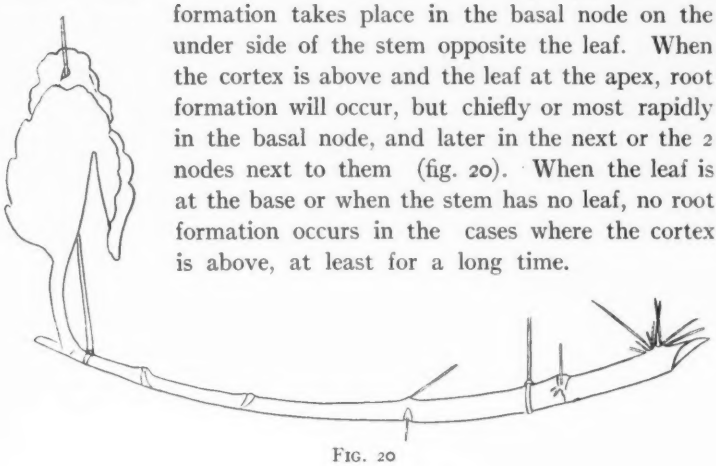

FIG. 20

IV. Mechanism of geotropic curvature in Bryophyllum calycinum

These experiments with split stems were used to obtain a more definite idea concerning the mechanism of the geotropic bending.

Immediately after the stems were split, marks were made with India ink on the cortex at a distance of 1 cm. from each other and then the stems were suspended horizontally, one-half of the split stems having their cortex below, the others having their cortex

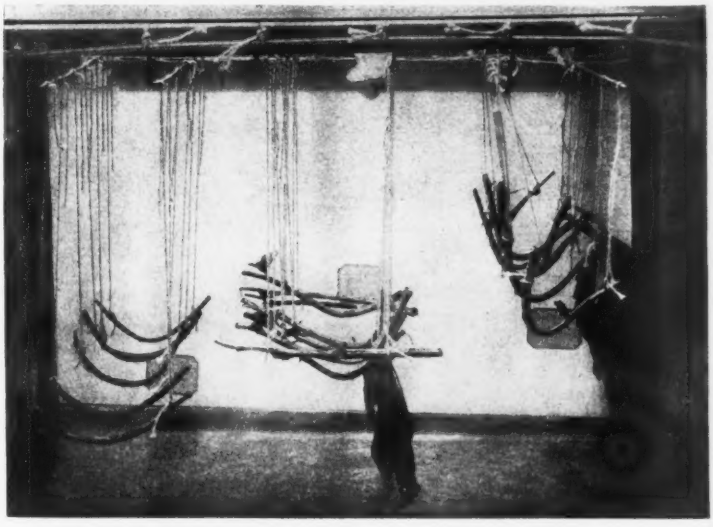

Fig. 21

above. Stems with an apical leaf were used for the purpose (like those in figs. 17, 20). After 10 days, when the halves with the cortex below had bent strongly, the displacement of the marks was ascertained. It was found that the marks on the halves in which the cortex was above and which had not bent were practically unchanged. The same was true of the marks in the non-bent regions of the other halves, where the cortex was below; while a growth of 15–20 per cent of the original length had taken place in the bent convex region of those stems having their cortex below.

Stems split lengthwise and with a leaf left at the most apical node were put horizontally into a jar saturated with water vapor. One-half of the stems were put with the cortex above (fig. 20), and one-half with the cortex below (fig. 17). Only the latter bent geotropically, the others showing only a slight concavity on the upper side, which may have been partly of a geotropic character, but which more likely was for the greater part, if not entirely, due

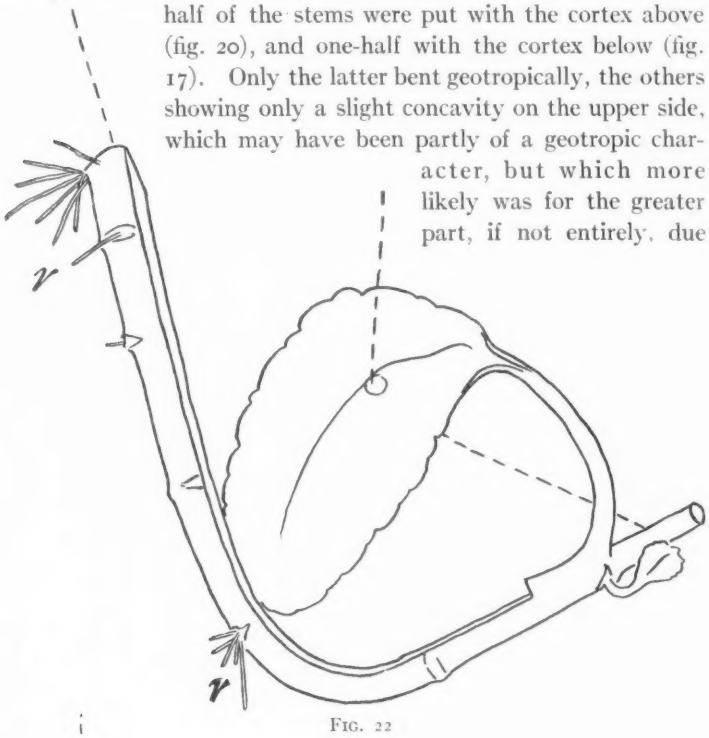

FIG. 22

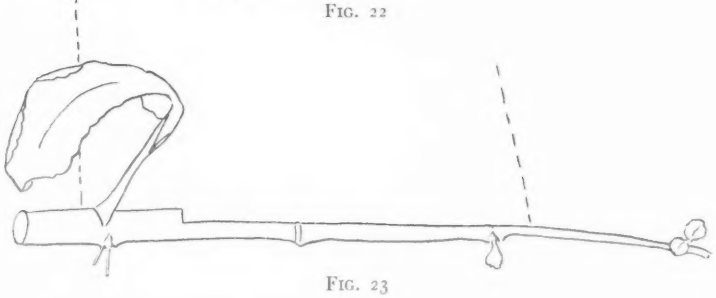

FIG. 23

to the tension of the cortex, which has a tendency to shorten in the longitudinal direction.

The experiment was begun June 21 and the measurements were taken on July 1. The original length of each piece of stem before the experiment was known from the India ink marks, the *final* length could be ascertained by direct measurement. First it was found that the length of the split stems which had been suspended with their cortex above was not altered, as shown in table I.

TABLE I

LENGTH OF SPLIT STEMS PLACED HORIZONTALLY WITH CORTEX ABOVE (IN CM.)

At beginning of experiment (June 20)	At end of experiment (July 1)
9.0	9.0
11.0	10.8
10.0	10.0
14.0	13.8

Obviously no growth had taken place in these halves; there may possibly have been a slight shortening, but if this was the case it was so small that it was within the limits of error of measurement.

An altogether different condition was found in the other halves of the stems which had been suspended horizontally with their cortex below. Here an increase in length was found in the bent part of the stem, while the apical and basal ends which had not bent were practically unaltered also in regard to length. We designate the apical unbent region *A*, the central bent region of the stem *B*, and the unbent basal region *C*. The measurements of 4 stems are given in table II (p. 40).

It is obvious that an increase in length of 15–20 per cent took place in 10 days in the bent central region of the stem (basally from or around the second node behind the apical leaf), while the unbent basal and apical regions showed no distinct alteration of length.

Fig. 24 is a photograph of marked *whole* stems 9 days after the beginning of the experiment. The stems had been suspended horizontally in the jar; all had one apical leaf left. That part of the cortex which was below had stretched, while the cortex above was shortened. The India ink marks were 1 cm. distant and were made at the beginning of the experiment. The photograph shows the change in the position of the marks on the convex and concave sides in the bent region of the stem.

It is highly probable, if not certain, that the increase in length on the lower side of the horizontally placed stem takes place primarily in the cortex of the bending region and not in the pith or wood. This follows from the behavior of these 2 parts when the cortex of a bent (split or whole) stem is removed, and the rigidity of the cortex is compared with that of the pith and wood taken out.

TABLE II

Region of stem measured	Stem I		Stem II		Stem III		Stem IV	
	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment	Beginning of experiment	End of experiment
A: non-bent apical part.....	3.0 cm.	3.2 cm.	3.0 cm.	3.0 cm.	4.0 cm.	4.0 cm.	4.0 cm.	4.1 cm.
B: bent central part.....	4.0	4.9	5.0	5.7	6.0	7.0	4.0	4.85
C: non-bent basal part....	2.0	2.0	3.0	3.0	5.0	5.0	4.0	4.15

If we remove the cortex on the lower (convex) side of a split geotropically bent stem, like that in fig. 17, we find that the rigidity of the cortex in the bent region is much greater than that of the wood or pith; the latter appears soft in comparison with the cortex of the bent region on the convex side of a geotropically bent stem. It is possible also that the increase in the rigidity of the cortex in this region may be due to a thickening of the cortex, a point which needs further investigation. Whatever the cause of this increase in rigidity may be, we reach the following conclusion regarding the mechanism of the geotropic bending of a horizontally suspended stem of *Bryophyllum calycinum*.

On the lower side of such a stem in a region the location of which depends upon the presence or absence, and, in the former case, upon the location of the leaf in the stem, the cortex begins to grow in length (and possibly in thickness). The wood, pith, and cortex on the upper side undergo no such growth. This increase in length (in one region) of the cortex on the lower side leads to a bending of the stem in which the lower side of a horizontally suspended stem becomes convex, the upper side concave.

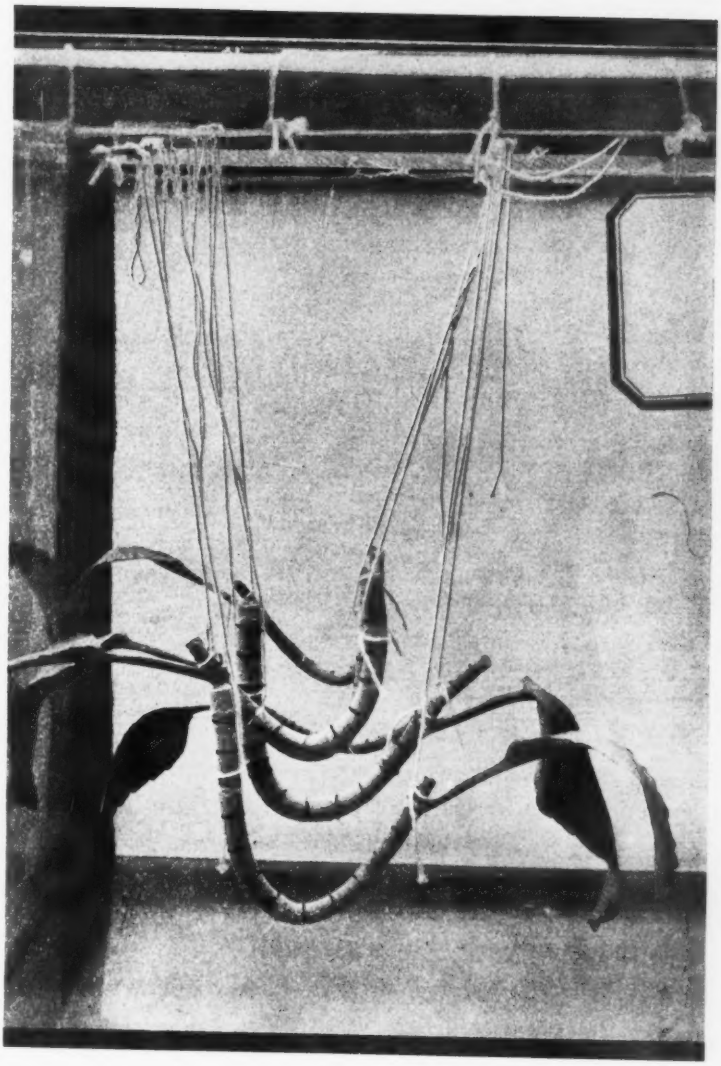

FIG. 24

Our investigation shows that this growing region of the cortex coincides with the region where early roots are formed. This suggests the possibility *that the geotropic growth of the cortex on the lower side of a horizontally suspended stem is due to a cause which is either closely associated or identical with the cause of root formation.*

If we assume with SACHS that there are specific root-forming substances, then the question presents itself whether we are not forced also to ascribe the geotropic curvature to the existence of specific geotropic substances or hormones; both substances having the tendency to collect on the lower side of a horizontally suspended stem; and both substances stimulating growth, the one of roots, the other of the cortex. On the basis of such an assumption we might understand why no or only an insignificant geotropic curvature takes place in a split stem when the cortex is on the upper side, the reason being that the geotropic substances settling at the lower side find no cortex which can grow and cause geotropic bending. This assumption will of course be a mere hypothesis until the existence of such hormones can be demonstrated directly.

V. FURTHER EXPERIMENTS ON THE INFLUENCE OF THE POSITION OF THE LEAF UPON THE GEOTROPIC BENDING OF A STEM

When we remove the cortex on the upper or lower side of a horizontally suspended stem of *Bryophyllum calycinum* (without removing the wood and pith), an extensive bending of the stem takes place instantly (fig. 25), the side on which the cortex is removed becoming convex. The mechanism of this phenomenon becomes clear on the assumption that the cortex is under a tension longitudinally which shortens the wood and pith. If this tension is removed on one side of the stem, the wood and pith on that side can stretch, while the wood and pith on the opposite side are held in check by the cortex. This leads to a considerable curvature whereby the side on which the cortex is preserved becomes concave (fig. 25). This curvature due to cortex tension is much stronger than the curvature which takes place instantly when we split a stem longitudinally. In this case not only the cortex but also the wood and pith are removed on one side of the stem, and hence the tendency of this side to stretch is considerably less than if only the cor-

tex is removed on one side. In the former case the stretching force of wood and pith on the side where the cortex is removed is lacking. Such stems show in a striking way the influence of the position of the leaf upon the geotropic curvature.

More than a dozen stems whose cortex was removed on the upper side were suspended horizontally (figs. 26, 27). Each stem had one leaf left, one-half of the stems having the leaf at the base (fig. 26), the other half having it at the apex (fig. 27). Only the latter bent geotropically, while the stems with the leaf at the basal end, not being able to overcome the resistance of the upper side of the pith and wood, did not undergo any geotropic bending. This shows that the geotropic growth of the cortex must be considerably less when the leaf is at the base than when it is at the apex.

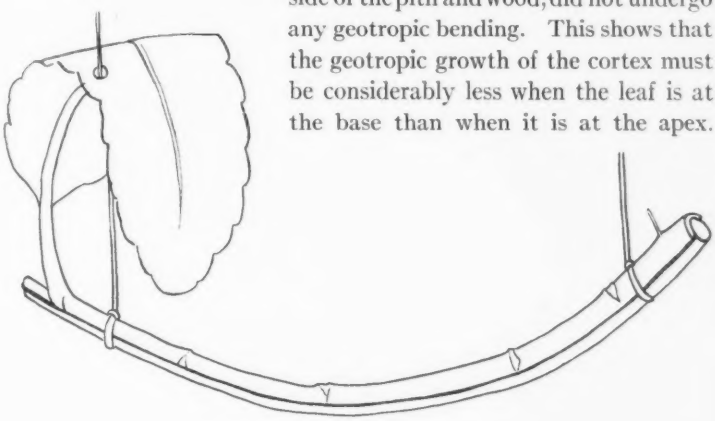

FIG. 25

When the cortex was above (fig. 25) and the leaf at the apex (or at the base), no or very little geotropic curvature ensued beyond the curvature due to the effect of the removal of the cortex on one side of the stem, which takes place instantly after the operation.

When the leaf is left at the apical end and the cortex below, as in fig. 27, the curvature occurs again in the region of the second node behind (basally from) the leaf; and in that node on the lower side the first roots develop (fig. 27). These drawings were made 9 days after the beginning of the experiment. If all the leaves are removed on such a stem it is no longer able to bend geotropically.

VI. FURTHER VARIATION OF THESE EXPERIMENTS

It is well known that the so-called geotropic "stimulus" goes around a corner, that is, around an incision. If we assume that the so-called "stimulus" is the flow of a liquid, we need not be surprised that it is able to go around a corner or around an incision in a stem. In a former paper (see footnote 1) we have shown that the "inhibition" of the stem upon the growth of the notches of a

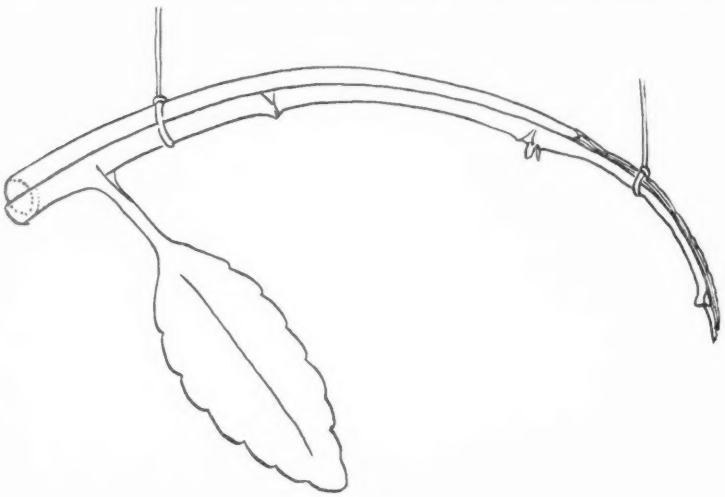

FIG 26

leaf also goes around a corner in a leaf when incisions are made into such a leaf; and the mysterious character of the phenomenon disappeared with the recognition of the fact that the "inhibition" is the flow of certain substances (water or solutes) from the leaf into the stem through a system of interlinked channels which allows a flow in a zigzag around incisions. The same conception will explain in our opinion why a geotropic "stimulus" will flow around an incision in a stem, the "stimulus" like the "inhibition" being the flow of certain substances through the leaf or stem respectively.

Incisions were made into each internode of stems of *Bryophyllum calycinum*, at *a*, *b*, *c*, and *d* in figs. 28 and 29. The stems were suspended horizontally in a jar saturated with water vapor. Six

stems had one leaf at the apex and below (fig. 28), the other 6 had 2 leaves at the basal node (fig. 29). All of the stems with the leaf at the apex bent geotropically (though not as rapidly as stems without incisions), while those with the leaves at the base remained unbent. Figs. 28 and 29 show the difference after 10 days. In

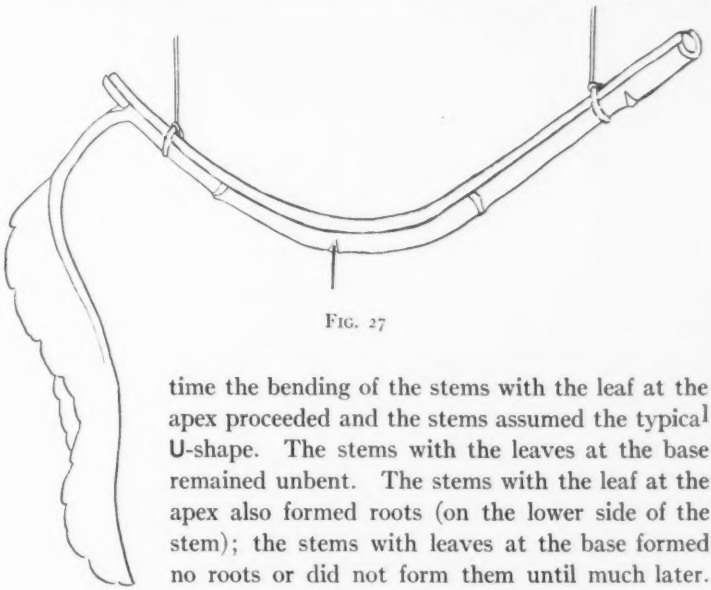

FIG. 27

time the bending of the stems with the leaf at the apex proceeded and the stems assumed the typical U-shape. The stems with the leaves at the base remained unbent. The stems with the leaf at the apex also formed roots (on the lower side of the stem); the stems with leaves at the base formed no roots or did not form them until much later.

VII. FORMATION OF ROOTS IN PASSIVELY BENT STEMS

We have seen that in stems suspended horizontally the roots have a tendency to form on the under side in the same region where the bending occurs. They form also at the basal nodes, both the upper and lower, but this fact does not concern us in this connection. The tendency of the roots to form on the lower side in that region which becomes convex might suggest the possibility that the root formation occurs in the convex region, not because it is the lower side, but because the convexity in itself might in some way favor root formation. The following experiment shows that the roots form on the lower side of a stem regardless of whether this lower side is concave or convex.

Stems were bent passively and fixed in this bent position by tying their ends to a piece of wood (fig. 30). Such pieces were then suspended in a jar saturated with water vapor. The experiments were made a year ago, before the writer was aware of the influence of the position of the leaf upon geotropic curvature and root formation, and in the expectation that passive bending of a stem would lead to the production of roots on the convex side of the stem. This was found not to be the case. Fig. 30 shows that numerous

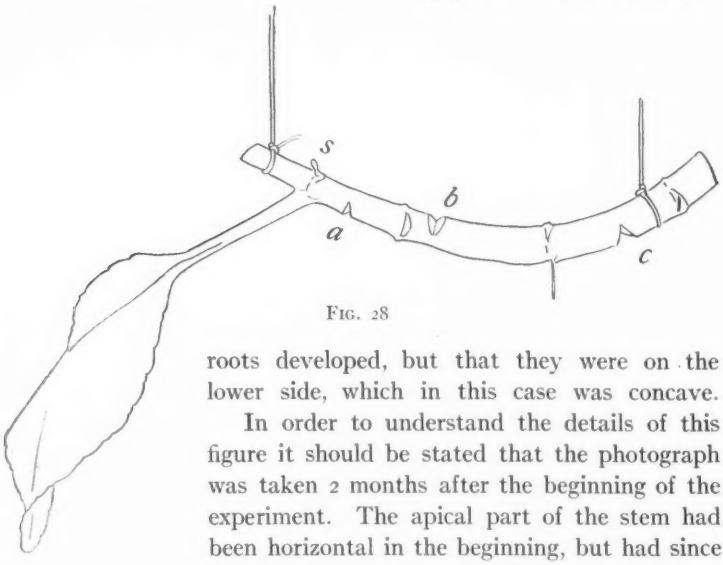

FIG. 28

roots developed, but that they were on the lower side, which in this case was concave.

In order to understand the details of this figure it should be stated that the photograph was taken 2 months after the beginning of the experiment. The apical part of the stem had been horizontal in the beginning, but had since bent upward, the bending taking place behind the second node. The side on which the roots were formed, therefore, had originally been the under side. The roots formed all along the lower side of that part of the stem which at first was in a horizontal position. Besides the small apical leaves, a large older leaf had been left on the stem, and from this leaf the stem was suspended. At the base of this large leaf and of the next 2 nodes a strong root formation took place. This is what we should expect on stems in which a leaf is left on the upper side.

Fig. 30 also illustrates in another way the influence of gravitation on root formation. The reader will notice that in the

horizontal region of the stem (basally from the large leaf from which the specimen was suspended) 2 large roots grew out from the internode. This occurs only after a long time and only on the lower side of a stem.

The experiment demonstrates, therefore, that roots will form on the concave side of a passively bent stem of *Bryophyllum calycinum* if this side is the under side of such a stem.

VIII. Theoretical remarks and summary

1. The theoretical remarks may be brief. We believe that these experiments show first that in *Bryophyllum calycinum* the substances which induce root formation have a tendency to collect on the lower side of a horizontally placed stem, although roots may appear also in nodes on the upper side (especially at the basal nodes), under special conditions which will be discussed in another paper. It is shown in this paper that a horizontally suspended stem of *Bryophyllum* will become concave on the upper side, and that this curvature, which will give such a piece a U-shape, is due to a longitudinal growth of the cortex on the under side of the horizontally suspended stem.

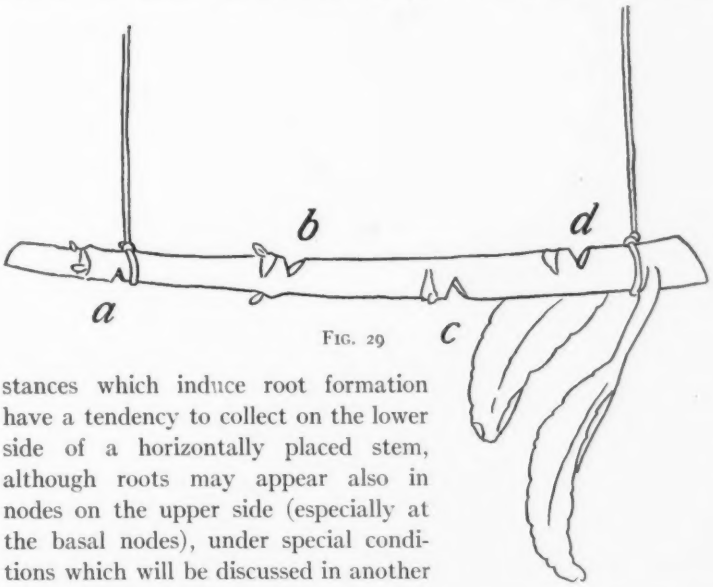

FIG. 29

2. We have seen that a leafless stem bends much more slowly than a stem in which one or more leaves are preserved; and we find

also that the roots form more slowly in a leafless stem than in a stem with leaves. We find also that in a general way the

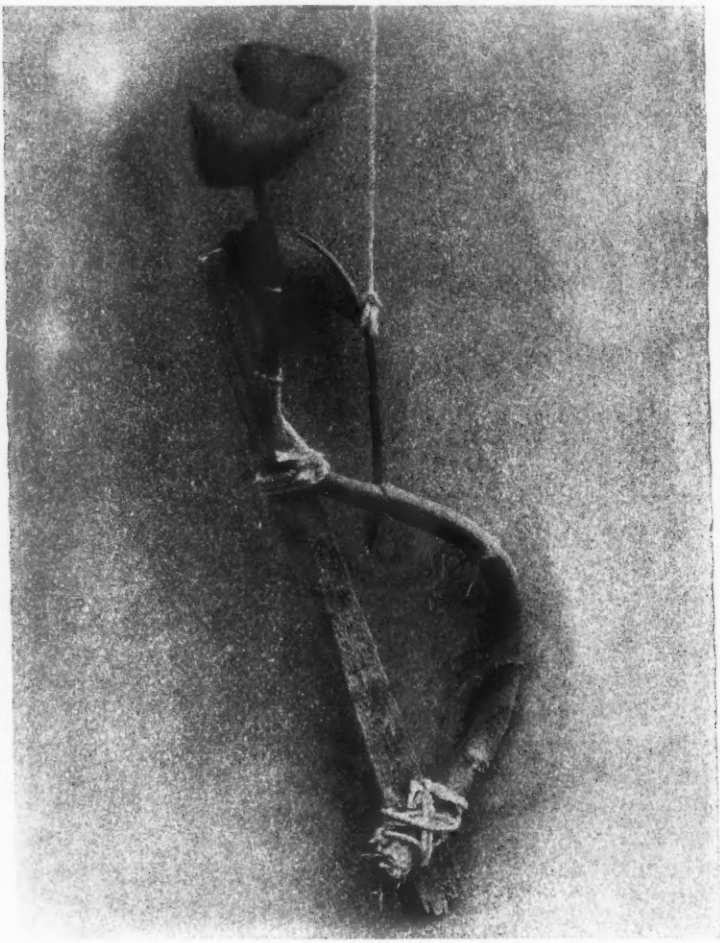

FIG. 30

amount of curvature and the amount of root formation vary in the same sense.

3. Both phenomena of geotropic curvature as well as of root formation depend in a striking way upon the position of the leaf on the stem. If in a horizontally suspended stem of *Bryophyllum* (in which the growing point is cut off) one leaf is preserved at the apex, and on the lower side of the stem, a rapid and very extensive geotropic curvature of the stem will take place, which is localized in the region basally from or around the second node from the leaf. The curvature is so extensive that the stem will assume the shape of a U with the concave side above. In such stems an extensive and rapid root formation will take place first in the second and fourth nodes behind the leaf on the lower side and also in the most basal nodes. The second and fourth nodes behind the leaf are therefore the centers of both kinds of growth in such a stem, namely, of the cortical growth which leads to the geotropic curvature and of the growth of roots. It should be pointed out also that in *Byrophyllum* the axes of successive nodes are always at right angles to each other, so that the favored nodes, the second and fourth behind the most apical one, all have the same orientation. It is quite possible that this structural peculiarity accounts for the fact that both root formation and geotropic curvature center around the second and fourth nodes behind a leaf left in the apex of an otherwise leafless stem.

4. If in a horizontally suspended stem only one leaf is left at the base of the stem (and on the lower side) the curvature is usually considerably less than in a stem with a leaf in the apex. The curvature in a stem with a basal leaf is confined to the region behind or around the leaf. It harmonizes with our previous statements that in such stems little or no root formation takes place, and that the root formation which occurs is confined to the node opposite the basal leaf and to the basal cut surface. When the piece of internode left behind the basal leaf is long, a more extensive curvature may occur than when the piece of internode left is short.

5. This difference in the influence of the apical and basal leaf can be made more striking when either the flow of substances in the stem is retarded (for example, by incisions in the stem) or when the resistance to the bending is made greater (by removing the cortex on the upper side of a horizontally placed stem whereby the

latter becomes concave on the lower side). In such cases geotropic curvature becomes possible only in stems with a leaf at the apex, but not in stems with a leaf at the base.

6. All these experiments become intelligible on the assumption that each leaf has a tendency to send shoot-forming substances toward the apex and root-forming substances toward the base of the stem. If it could be proved that in *Bryophyllum calycinum* a specific substance (hormone) is responsible for the geotropic growth (in the cortex of the lower side of a horizontally suspended stem), we might say that both substances show a tendency to collect on the lower side of a horizontally placed stem, and that the flow of both is influenced in the same way by the leaf. The apical leaf sends both substances toward the base of a stem, while the basal leaf acts as if it had a suction effect upon geotropic substances contained in the apical region. Such an idea suggests itself from the fact that a leafless stem has the center of its geotropic curvature in the middle (fig. 15), while a stem with a leaf at the base has either no curvature (fig. 16) or has it only in the region of the leaf.

While in *Bryophyllum* the hypothetical geotropic hormone is associated (or identical) with the root-forming hormone, in other plants the hypothetical geotropic substance might be associated with the shoot-forming hormone. This would explain the fact that in certain fir trees a horizontal branch next to the apex may suddenly become negatively geotropic when the apex is cut off. After the decapitation the (hypothetical) geotropic substance which before was flowing to the apex now can flow into the horizontal branches next to the apex, and the one which by chance gets a little more of the substance than the others will be the first to become vertical. After this the mechanical advantage due to the vertical position will favor the continued flow of these substances in this branch, which thus becomes the new apex.

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NEW YORK CITY

PROTHALLIA AND SPORELINGS OF THREE NEW ZEALAND SPECIES OF LYCOPODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 222

CHARLES J. CHAMBERLAIN

(WITH PLATES II–III)

Few botanists have even seen the prothallia of *Lycopodium*, and most of those who have had such a privilege are indebted to one man, BRUCHMANN of Gotha; fewer still have made any investigation of the subject. The reason prothallia and sporelings of *Lycopodium* have not been so extensively studied as those of the ferns is not lack of interest, but difficulty in germinating the spores or finding prothallia growing naturally. In 1911 I collected adult plants of several New Zealand species of *Lycopodium* and made some effort to find prothallia, but my time was too limited for such slow, uncertain work. However, Professor A. P. W. THOMAS, at that time botanist of the University at Auckland, very kindly gave me prothallia and sporelings of 3 characteristic New Zealand species, *Lycopodium laterale*, *L. volubile*, and *L. scariosum*. Prothallia and sporelings are known in so few species that it seems worth while to give some account of this material.

Since the literature of the subject was examined with considerable care, all of the original papers cited being available, and since E. A. SPESSARD, a student of mine, is announcing in this issue of the BOTANICAL GAZETTE the first discovery of the prothallia and sporelings of several American species of *Lycopodium*, a historical résumé may be of service to those who are in favorable localities and who might wish to study the subject.

Historical

TREUB (**7**) introduced his classic account of the prothallium of *Lycopodium cernuum* with the remark that the history of the subject could be given in a few words, since it was necessary to cite only 3 or 4 investigators. SPRING (**2**), HOFMEISTER (**3**), DEBARY (**4**), FANKHOUSER (**5**), and BECK (**6**) are mentioned; but neither SPRING

nor HOFMEISTER ever saw any prothallia. DEBARY, and later BECK, germinated the spores of *L. inundatum*, and FANKHOUSER found a few prothallia of *L. annotinum*.

TREUB overlooked the English surgeon, JOHN LINDSAY (1), who nearly 100 years before had germinated the spores of the very species with which TREUB was dealing. LINDSAY was also the first to raise ferns from the spores. Having noted very young ferns growing in the open in Jamaica, he sowed the spores ("farina") and watched their development. It was in connection with this work that he tried *L. cernuum*. His own account is interesting: "I have very lately sown that fine farina or dust contained in the anthers of a species of the genus *Bryum*, namely, *Bryum caespititium*, or one very like it, and also the farina of *Lycopodium cernuum*. There is a vegetable growth taking place where they were sown which I hope will prove to be their young plants." Later, in a letter to Sir JOSEPH BANKS in regard to *Bryum caespititium* and *Lycopodium cernuum*, he states that he had repeatedy sown them both, and in proper situation found that they grew very readily. There were no further figures or descriptions of either the *Bryum* or *Lycopodium*.

SPRING's failure to germinate spores brought him to the curious conclusion that *Lycopodium*, and also *Psilotum* and *Tmesipteris*, consist exclusively of male plants, the females having been destroyed in some geological catastrophe. At this time it was generally believed that the spores, if they should germinate, would develop directly into the leafy plants. A few years later, HOFMEISTER opposed this view and predicted that the spores would give rise to prothallia bearing antheridia and archegonia, and that the leafy plant would arise from the fertilized egg; but all attempts to prove his theory by germinating the spores resulted in failure.

DEBARY succeeded in germinating the spores of *L. inundatum*, and in 9 days obtained prothallia consisting of 7 cells; but repeated attempts failed to produce more advanced stages, except that one prothallium was observed which had reached the 11-cell stage. The prothallia soon died.

In 1873, FANKHOUSER, in Switzerland, found prothallia and sporelings of *L. annotinum* growing naturally. This fortunate find,

together with the results of DEBARY, who had described early stages of *L. inundatum* from cultures, made it possible to make a general outline of the development from the germination of the spore up to the adult prothallium with sporelings attached.

BECK sowed the spores of various species under various conditions, but *L. inundatum* was the only one to germinate and the prothallia did not get beyond the 10-cell stage. He asserted that after 2 years the spores of *L. clavatum* showed chlorophyll and looked as if they were about to germinate, but no cell division occurred.

Since FANKHOUSER'S paper appeared, 3 men have made large contributions to our knowledge of these peculiar prothallia. In 1884, 1886, 1888, and 1889, TREUB (**7, 9, 11, 12**) published a splendid series of researches upon the prothallia of Javanese species. In 1885, BRUCHMANN (**8**) began his patient and persistent researches upon the difficult temperate species with subterranean prothallia which had baffled all previous investigators; and in 1887, GOEBEL (**10**) found prothallia of *L. inundatum*, so that, with the stages secured by DEBARY, he was able to give a connected account of this species.

TREUB began his series with an investigation of *L. cernuum*. He germinated the spores of this familiar tropical species and some of the prothallia reached the early antheridium stage before they died. However, he found abundant material growing wild, and so had a complete series from the germination of the spore to the adult prothallium with embryos and older sporelings.

The prothallia are green and grow on the surface of the substratum, the largest reaching a height of 2 mm. When the spore germinates, a more or less spherical body is formed, about 8 or 10 cells in diameter. From the top of this body, which TREUB called the "primary tubercle," an alga-like filament then develops, at first consisting of a single row of cells, but soon dividing in all planes, so that a stout cylindrical body is formed more than twice the diameter of the primary tubercle and 5 or 6 times as long. The tip of the cylindrical portion is profusely branched, and at the base of the branches the antheridia and archegonia are borne. The embryogeny is particularly interesting, since the fertilized egg does

not develop directly into a leafy plant, but produces a protocorm with protophylls, resembling a miniature *Phylloglossum*.

TREUB (9) next dealt with *L. Phlegmaria*, another familiar tropical species, epiphytic upon trees. The prothallia are found on the tree trunks just below the surface of the humus, but they have no chlorophyll, being entirely saprophytic and abundantly supplied with an endophytic fungus. The main body is tuberous, about 2 mm. in diameter, more or less spherical or somewhat elongated, and has several branches extending in various directions. The branches vary from 1 to 6 mm. in length and bear antheridia or archegonia or reproductive bodies which TREUB called "propagula." The antheridia and archegonia are usually at the tips of the branches, not at the base as in *L. cernuum*. The propagula are of two general types, one consisting of scores or even hundreds of cells forming a flask-shaped body with a slender stalk of one or two cells; the other is much smaller, more or less spherical in shape, and consists of only a few cells, usually not more than 2 or 3, with the outer walls very much thickened. In the first type the propagula break off at the stalk and grow directly into new prothallia, while in the second type there is a more or less prolonged resting period. The first type seems to correspond to the gemmae of liverworts and mosses, and the second type seems to correspond to the brown bulbils of mosses.

TREUB was not able to germinate the spores, and he believed that most of the prothallia found in nature come from propagula, prothallia from spores being comparatively rare.

The accounts of the development of antheridia, archegonia, and embryo are very complete, but the vascular anatomy of the sporeling is not described.

The prothallia of *L. salakense* are aerial, green, have no endophytic fungus, and are the only ones which TREUB succeeded in raising from the spore to the adult stage with antheridia and archegonia. In a few days the prothallia developed to the primary tubercle stage, then rested for several months, and finally resumed their growth and completed the life history. As in *L. cernuum*, a simple filament appears at the top of the primary tubercle and then forms a cylindrical body several cells in thickness, but other fila-

ments then develop and behave in the same manner, so that there are several branches. The antheridia and archegonia are formed at the tops of these branches, there being no leaflike organs as in *L. cernuum* and *L. inundatum*.

The prothallia of *L. carinatum*, *L. Hippuris*, and *L. nummulariforme* are all of the *L. Phlegmaria* type, those of *L. carinatum* bearing such a close resemblance that TREUB (**11**) warns prospective collectors against collecting in localities where both species occur, since it is impossible to distinguish either the prothallia or the embryos. The prothallia of *L. Hippuris* are similar, but are more vigorous and the branches are thicker. TREUB was not able to disentangle complete prothallia of *L. nummulariforme* from the substratum and so had to write his account from fragments. He did not find any endophytic fungus.

The final paper in TREUB'S (**12**) series dealt with the embryo of *L. cernuum*. The series of stages was very complete, from embryos consisting of a few cells, through the protocorm stages, and up to sporelings with a few leaves. After the embryo has developed a protocorm with protophylls resembling a small *Phylloglossum*, a definite growing point is organized which develops into a leafy axis and at the same time the first root appears. TREUB indicated the course of the vascular bundles, but did not make any further study of the anatomy. He regarded the protocorm as a recapitulation of a *Phylloglossum* stage in the ancestry of *Lycopodium*.

In 1884, the same year that TREUB (**7**) began his research upon tropical forms, BRUCHMANN (**8**) found 3 prothallia of *L. annotinum*, and thus began a series of researches which extended over 25 years and resulted in clearing up the life-histories of the much more difficult temperate species. BRUCHMANN'S first paper appeared in 1885, but TREUB'S first account, although dated 1884, appeared at about the same time, so that neither knew the other was working upon prothallia. BRUCHMANN'S (**13**) most extensive work, which gained him the prize of the Paris Academy of Science, appeared in 1898, and contained descriptions of *L. clavatum*, *L. annotinum*, *L. complanatum*, and *L. Selago*. All were found growing in the Thuringer forest near Gotha, but the germination of the spores and earliest stages in the development were lacking. The development

of antheridia, archegonia, and embryos are very clearly described. Ten years later this account was supplemented by a very complete description of *L. complanatum*. Although BRUCHMANN (**15**) had made repeated efforts to germinate the spores of various species, he met only the failures which had discouraged other botanists; but finally his perseverance was rewarded and he was able to give a complete account of the germination and early development of *L. clavatum*, *L. annotinum*, and *L. Selago*. The surprising feature is the long-delayed germination. The spores of *L. Selago* germinated in 3–5 years, and the development of antheridia and archegonia was complete in 6–8 years; *L. clavatum* and *L. annotinum* were even slower, germinating in 6–7 years and requiring 12–15 years to complete the development of antheridia and archegonia. BRUCHMANN suggests that possibly the periods might be shortened artificially if the proper stimuli could be discovered. All the species reported in BRUCHMANN'S various papers are subterranean and saprophytic, but the spores germinate independently and develop to the 4 or 5-cell stage, and at this stage the fungus must enter or there will be no further development.

L. salakense, which TREUB (**11**) succeeded in keeping in cultures throughout the whole life history, is green, aerial, has no fungus, and germination takes place in a few days. *L. cernuum* is also aerial and green and germinates with equal promptness, but it does not develop beyond the primary tubercle stages unless the fungus enters. *L. inundatum* in DEBARY'S cultures developed to an early primary tubercle stage with some chlorophyll and then died. The subsequent work of GOEBEL (**10**), who found prothallia growing naturally, proved that this species also has an aerial, green prothallium with an endophytic fungus. In *L. cernuum* and *L. inundatum*, however, the fungus infection is much slighter than in the saprophytic species.

So far as I have been able to determine, there is only one paper which makes any mention of the prothallia of New Zealand species of *Lycopodium*, and this paper by HOLLOWAY (**16**) deals primarily with the anatomy of the sporophyte. The investigation, both in the field and in the laboratory, is of such high grade that we hope HOLLOWAY will sometime give us an extended account of the pro-

thallium and the anatomy of the sporeling. The varied species, ranging from epiphytes to ground forms, with prothallia ranging from the green, leafy aerial type to the deepest subterranean tuberous type, make New Zealand an ideal place for such a study.

Material and observations

The epiphytic type is not represented in the species at my disposal. *Lycopodium laterale* has a stout creeping rhizome, with numerous erect branches, and cones borne laterally; *L. scariosum* has a somewhat similar habit, except that the cones are terminal; *L. volubile* is the most beautiful species of the genus, bearing a striking resemblance to *Selaginella* as it trails along the ground or over bushes; but, unlike *Selaginella*, it keeps well after being gathered and is much used for table decoration.

PROTHALLIA

L. LATERALE.—The only reference I have been able to find in regard to the prothallium of this species is in HOLLOWAY's (16) paper. He says "in the case of *L. laterale* prothallial plants were found in two localities, growing on recently overturned marshy soil. The prothallus of this species corresponds to the type of *L. cernuum*, is small and short-lived, and is situated at the surface of the ground."

I had at my disposal 3 prothallia with protocorms attached and one older protocorm entirely free from the prothallium. In the first 3, each of the protocorms bore 2 fully grown protophylls; the older protocorm bore 10 protophylls. Two of the prothallia with their young plants are shown in figs. 1 and 2, the exact size being indicated in fig. 1*a*. The older protocorm with its 10 protophylls is shown in fig. 3. In fig. 1 the particles of sand and soil are not represented.

The upper half of the prothallium projects above the surface of the soil. There is a more or less spreading crown of leafy lobes, abundantly supplied with chlorophyll, and at the base of the inner face of these lobes the antheridia and archegonia are borne. It seems evident that the base of the prothallium was first to develop, but no sharply differentiated primary tubercle, like that shown in

TREUB'S figures of *L. cernuum*, was found in these specimens. However, the base of the prothallium is more pointed than in *L. cernuum*, and this pointed base may represent the primary tubercle.

In proportion to the size of the prothallium, the protocorm is much more massive than in *L. cernuum*. There is no single, definitely organized growing point giving rise to all the protophylls, but rather a series of points, each giving rise to a protophyll. Stomata are abundant almost to the base of the protophyll; they are of the simplest type and open into a loose parenchyma with large air spaces. The transverse section is circular and shows a single weak vascular strand extending a short distance into the protocorm and ending blindly, without uniting with the strands of neighboring protophylls. The protuberance shown in front of the large protophyll in fig. 2 might be mistaken for the growing point from which the leafy axis is to be developed, but that point is formed much later, after several protophylls have appeared. The prothallium and protocorm shown in fig. 2 are similar, but indicate that there is considerable variation in both structures. Outlined against the protocorm is a second embryo.

The much older protocorm (fig. 3) indicates that the protophylls arise at irregular points, although there is a general progression, so that the protocorm resembles a very short horizontal rhizome. The 2 protophylls in the foreground are evidently the first ones formed, and the 3 much smaller ones at the left are the latest. The leafy axis of the permanent plant has not yet appeared. This specimen and also those shown in figs. 1 and 2 were sectioned, but the soil prevented satisfactory results. However, the sections showed the position of sex organs, the distribution of the fungus, and the relation of the protocorm to the prothallium. These features are shown, in a very diagrammatic way, in fig. 1*b*.

L. laterale belongs definitely to the type represented by *L. cernuum* and *L. inundatum*, since it has a short-lived green prothallium and an ephemeral protocorm with protophylls preceding the permanent leafy plant. *L. salakense* also belongs here, since the prothallium is green, and in its earlier stages behaves like that of *L. cernuum;* but it differs from the other 3 in having no endophytic

fungus. Whether it has a protocorm stage is not known. TREUB failed to find sporelings when he made his first investigation; later, in his work on the embryogeny of *L. cernuum*, he figures a protocorm stage in *L. salakense*.

It is interesting to note that the protocorm stage has been found only in *L. cernuum*, *L. salakense*, *L. inundatum*, and *L. laterale*, all of which have spores which germinate, giving rise to green short-lived prothallia. The spore-bearing plants of all 3 species, as well as that of *L. salakense*, grow upon the ground. No green prothallium or a protocorm phase in the embryogeny has yet been reported for any epiphytic *Lycopodium*.

L. VOLUBILE.—The only reference to the prothallium of this species is by HOLLOWAY (**16**). He says "the prothallus is large, firm, and long-lived. Healthy prothalli were seen still attached to sporelings which were as much as 10 cm. in length. Generally the prothalli are subterranean, being buried 1–4 cm. in depth; in several instances, however, they were observed growing on the surface of the ground, and the upper portion of the prothallus was then well supplied with chlorophyll."

The material at my disposal included 9 prothallia and 2 sporelings, one of them still attached to the prothallium (figs. 4–9). All belong to the subterranean tuberous type, and 4 of them (figs. 4, 5, 8, and 9) show a primary tubercle. Although the material is somewhat limited, it is evident that there is considerable variation in size and form.

The endophytic fungus is most abundant midway between the center and the surface, and is entirely lacking in the crown, in the upper part of the depression within the crown, and in the axis of the prothallium. Cells with considerable fungus abut directly upon those with none at all, making a sharp contrast (fig 12). A detail is shown in fig. 13.

The crown is differentiated into two regions in some places, only the inner one of which bears archegonia and antheridia, as shown in figs. 5 and 6; but even in these 2 prothallia some portions of the crown show no such differentiation, and the prothallia shown in figs. 7 and 8 have uniformly rounded crowns with no indication of two regions. While most of the sex organs are on the swollen rim

of the crown, they are not confined to this region, but occur in scattered patches within the rim on any part of the depressed region. A sectional view of a typical distribution of archegonia, antheridia, and the fungus region is shown in fig. 14.

The antheridia vary in size, shape, and output of sperms. They form hemispherical projections, with a nearly spherical mass of sperms; or they project scarcely at all, in which case the mass of sperms is not quite so regular. In a few cases, the sperm mass was elongated, making the topography bear some resemblance to that of an archegonium. In all cases, only one layer of cells separates the sperms from the surface, so that the essential course of development is uniform. A typical view is shown in fig. 15.

The foot of the sporophyte is strongly haustorial, and the cells surrounding it have some starch but very little protoplasm or other visible contents; consequently, the food supply must come largely from the fungus region and must be in a liquid condition even at a considerable distance from the foot. This is quite different from the condition in some gymnosperms, where only a single layer of cells may separate the haustorial cells of the embryo from those containing an abundance of food material in solid form. The foot is small and the vascular strand does not extend into it, but extends in an unbroken line from the shoot into the root, which is very late in developing. However, a few elongated cells, which do not become lignified, bend away from the main axis and point toward the foot.

L. SCARIOSUM.—The only description of this species is that given by HOLLOWAY (**16**), who says "the prothallus of *L. scariosum* was discovered in two localities. Like that of *L. volubile*, it appears to correspond to the *L. clavatum* type. It is large, firm, and long-lived, and in every case was found deeply buried (2–6 cm.)." Three specimens of this species were available and all had reached maturity, one bearing a young plant 18 mm. long, and the other two showing the foot and base of younger sporophytes which had broken off. Both prothallium and sporeling are larger and coarser than in *L. volubile*, as can be seen by comparing figs. 9 and 11, which are drawn to the same scale. The prothallium is densely infested by the fungus, which has about the same distribution as in *L. volubile*.

ORIGIN OF THE SUBTERRANEAN HABIT.—That the green leafy prothallia represent the original type from which the subterranean forms have diverged can scarcely be doubted. The species with green, leafy prothallia (*L. cernuum*, *L. inundatum*, *L. salakense*, and *L. laterale*) have spores which, in the first 3 species, are known to germinate immediately; while in all those with subterranean prothallia the spores germinate only after a long resting period. It would seem that some change has occurred in the spore which has delayed the germination; and then only such spores as reached a protected situation would survive to germinate at all. Germinating in protected situations, with little or no light, the prothallia naturally would assume the forms of subterranean, dependent structures. That this has been the order of regression is indicated by the fact that the leafy crown has not been lost altogether, but only modified. In *L. annotinum*, as described by BRUCHMANN, the prothallium is subterranean and saprophytic, but still retains some of the leafy appearance; in *L. laterale* the crown is sometimes broken up into separate fleshy cushions which may represent leafy lobes; in more extreme cases, there is merely a swollen, fleshy rim to represent the leafy structure. The position of antheridia and archegonia is about the same as in the green, leafy forms.

If those who are expert in hastening the germination of seeds which normally have a long resting period, could find some way to make the spores of *L. annotinum*, or some such species, germinate immediately, it would not be surprising if green, leafy prothallia should appear.

ANATOMY OF THE SPOROPHYTE

In the vascular structure of the adult sporophyte *Lycopodium* still presents some difficult problems, although investigations like those of HILL and others have cleared up some of the phases. However, it seems likely that the final solution will come through a comparative study of sporelings, intermediate stages, and adult plants. TREUB (**7**), BRUCHMANN (**13**), Miss WIGGLESWORTH (**14**), and HOLLOWAY (**16**) have figured and described a few sections; but material has been scanty or other features of the problem have so engrossed the attention that this important feature has received little attention.

It would be dangerous to draw any serious conclusions from a study of 2 or 3 sporelings, all of which had reached the leafy stage; but, in the present condition of the subject, it seems worth while to describe a few features. The study was made from the sporelings shown in figs. 9–11.

In *L. volubile* the foot is quite small, and although somewhat larger in *L. scariosum*, no vascular strand extends into it, but a few cells, not lignified, point in its direction. The vascular strand extends in an unbroken line from the tip of the stem to the tip of the root, which in both species is late in appearing.

The sporeling of *L. volubile* shown in fig. 9, and that of *L. scariosum* shown in fig. 10, were sectioned transversely down to the crown of the prothallium, and the portion below the crown was then cut longitudinally. It would have been much better if transverse sections had been continued throughout. In both species the leaves are surprisingly like the protophylls of *L. laterale*. Throughout a considerable portion of their length the transverse section is circular, and even in the broader middle region the leaves are thick and spongy, consisting almost entirely of very loose parenchyma with large intercellular spaces and a single vascular strand. Stomata are irregularly scattered over the entire surface (fig. 16). The adult leaves in both species are rather thin.

In *L. scariosum* the shifting topography of the stele is a conspicuous feature, especially in the upper, leafy region; in the lower half of the sporeling, where there are only a few scale leaves with no leaf traces, the arrangement is more uniform. Near the middle of the leafy portion, a hexarch, pentarch, and tetrarch condition occurs within a vertical distance of 1 mm. Throughout the lower one-third of this specimen the stele is rather constantly tetrarch; but, just above the foot a few sections show a triarch and even a diarch stele. That the leaf traces connect with the protoxylem points is evident at a glance; but whether the leaf traces determine the topography is not so clear. However, it is significant that the stele is more complex in the leafy region and that it attains its greatest complexity in mature plants with larger leaves and vigorous leaf traces. In various places there are indications of the banded arrangement characteristic of the adult stele.

The differentiation of the vascular tissues is interesting. A short distance below the meristem the large cells which are to form the largest tracheids are easily recognized, and some of the cells of the points of the radial structure can be distinguished, although lignification has not yet begun. Very soon the points of the radial structure begin to lignify and are then marked off very sharply from the surrounding tissues (fig. 17). These patches of lignified tissue consist almost exclusively of coarsely pitted tracheids. It is possible that there are some spiral vessels, but it looks as if practically all of the spirally marked cells belong to the leaf traces. If protoxylem is to be identified by spiral and annular markings, very little of the tissue which becomes lignified at this early stage would satisfy such a criterion; but the tissue is so well defined and becomes lignified so far in advance of the rest of the xylem, and is so sharply marked off from the large cells which in longitudinal view have the typical scalariform marking, that it may very properly be called the protoxylem. It should be recalled that in the cycads spiral vessels in the protoxylem are largely confined to the seedling, the protoxylem of the adult plant consisting almost exclusively of tracheids.

The study of the root was not satisfactory. Near the tip the bundle is C-shaped and diarch with the phloem in the sinus.

The sporeling of *L. volubile* is comparatively slender and in every way more delicate than that of *L. scariosum*. In the upper leafy portion the stele is quite regularly radial and tetrarch; but from the secondary root (*r* in fig. 9) down to the prothallium the structure is regularly or irregularly triarch. In connection with the more uniform topography of this stele, it should be noted that the leaves and their single vascular strand are not nearly so robust as in *L. scariosum*. The adult stele has the banded arrangement. The differentiation of the tissues of the stele proceeds as described for *L. scariosum*.

Summary

1. *Lycopodium laterale* has a green, leafy prothallium, and there is a protocorm-protophyll stage in the embryogeny. *L. volubile* and *L. scariosum* have subterranean prothallia with no protocorm stage, but the early leaves have the structure of protophylls.

2. In *L. scariosum* and *L. volubile* the sporeling has a radial stele. The adult plants have a banded stele.

3. The outer part of the ray of the radial structure consists almost exclusively of pitted tracheids with scarcely any spiral vessels, but becomes lignified long in advance of the large tracheids of the metaxylem, and should be regarded as the protoxylem.

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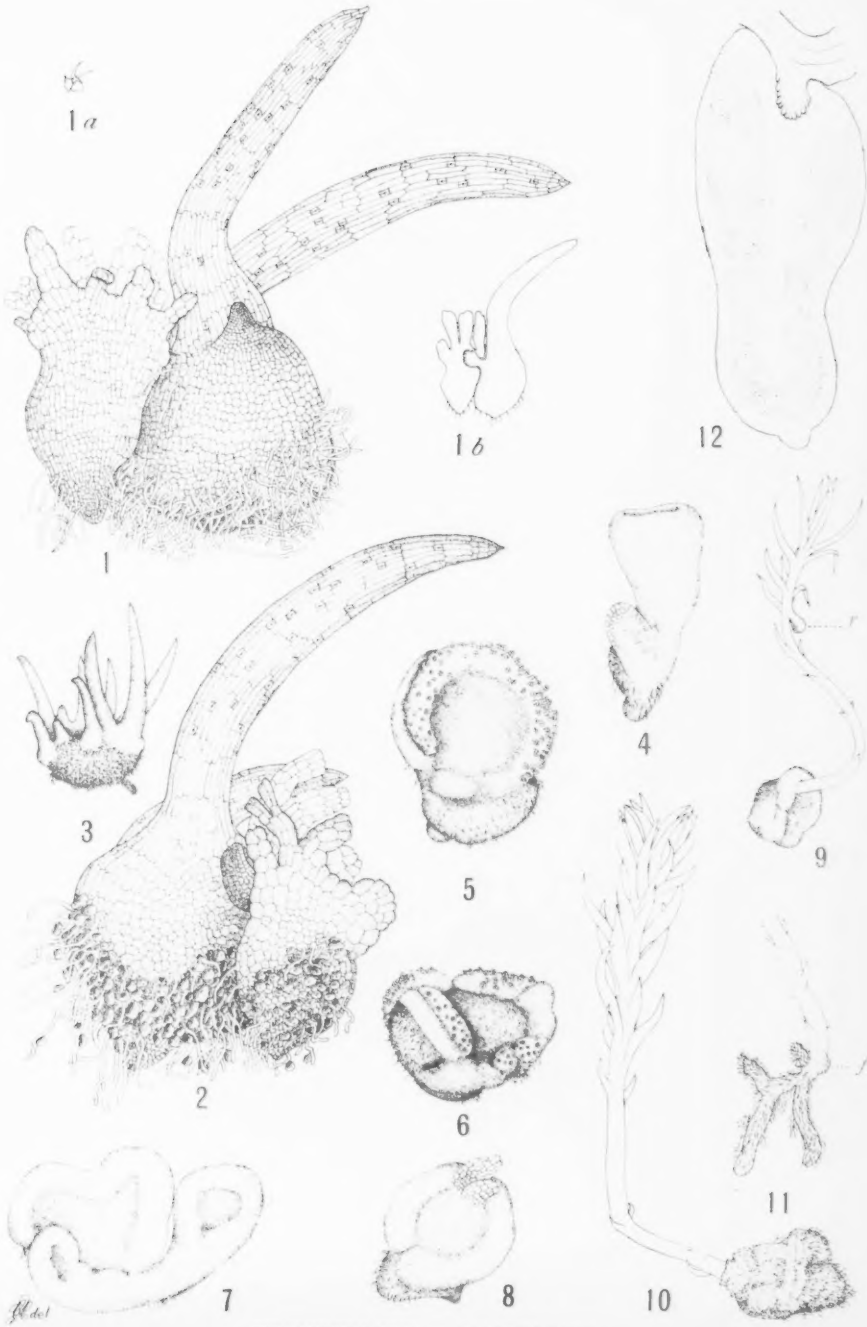

CHAMBERLAIN on LYCOPODIUM

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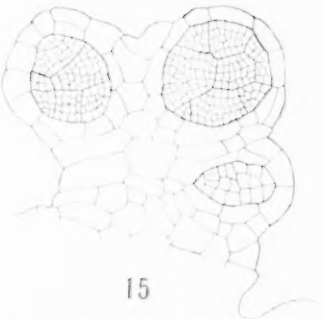

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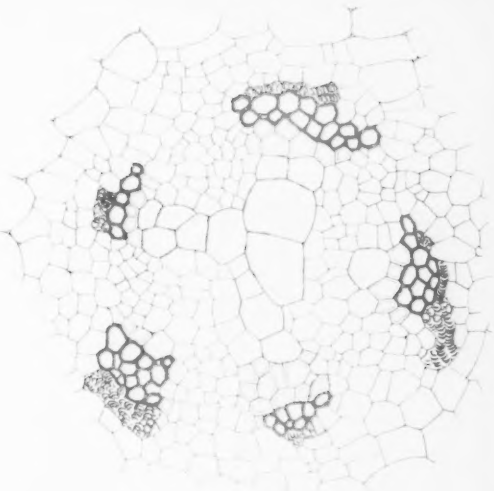

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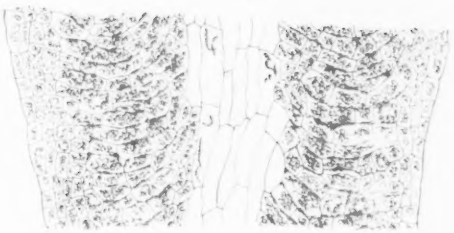

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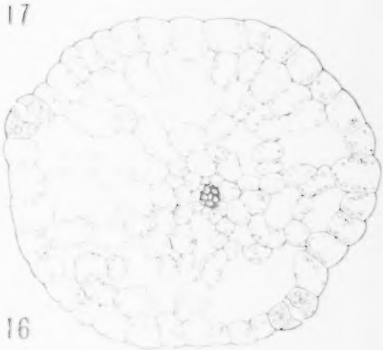

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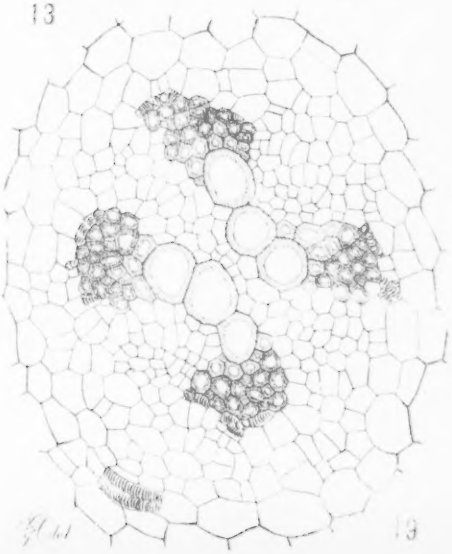

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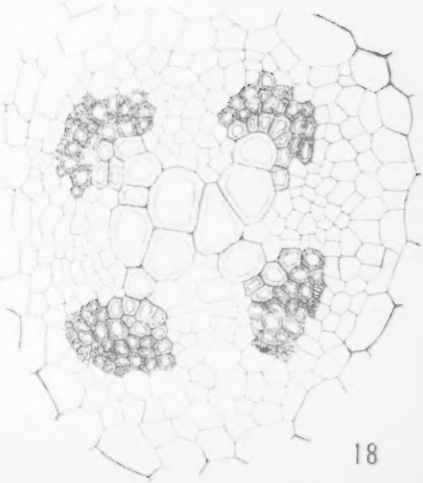

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EXPLANATION OF PLATES II–III

FIG. 1.—*Lycopodium laterale:* prothallium with leafy crown at left and bearing, at right, a protocorm with 2 fully grown protophylls and one young protophyll; ×20 (fig. 1*a* shows this plant natural size; fig. 1*b* is sectional view).

FIG. 2.—*L. laterale:* prothallium, at right, bearing a protocorm with 2 protophylls and also a second embryo; the soil and sand have not been removed; ×20.

FIG. 3.—*L. laterale:* protocorm with 10 protophylls; ×6.

FIG. 4.—*L. volubile:* prothallium showing primary tubercle at base; the crown is very even; ×10.

FIG. 5.—*L. volubile:* prothallium showing crown with numerous archegonia and antheridia; primary tubercle at base; ×10.

FIG. 6.—*L. volubile:* prothallium with archegonia and antheridia; the crown is lobed; ×10.

FIG. 7.—*L. volubile:* large, irregular prothallium; ×10.

FIG. 8.—*L. volubile:* prothallium with 2 young sporophytes; ×10.

FIG. 9.—*L. volubile:* prothallium with sporeling; a secondary root is shown at *r;* ×4.

FIG. 10.—*L. scariosum:* prothallium with sporeling; ×4.

FIG. 11.—*L. scariosum:* lower part of a young sporeling showing roots and foot; ×4.

FIG. 12.—*L. volubile:* diagrammatic view of prothallium with foot and lower part of sporeling.

FIG. 13.—*L. volubile:* detail of prothallium.

FIG. 14.—*L. volubile:* diagrammatic sectional view of prothallium showing archegonia, antheridia, and distribution of fungus, the latter indicated by shading.

FIG. 15.—*L. volubile:* portion of prothallium showing antheridia.

FIG. 16.—*L. volubile:* transverse section of leaf.

FIG. 17.—*L. scariosum:* transverse section of stem of sporeling before the large tracheids become lignified; *px*, protoxylem; *p*, phloem.

FIG. 18.—*L. scariosum:* same sporeling lower down; large tracheids have become lignified.

FIG. 19.—*L. scariosum:* same sporeling showing indication of banded arrangement.

PROTHALLIA OF LYCOPODIUM IN AMERICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 223

EARLE AUGUSTUS SPESSARD

(WITH TWENTY-ONE FIGURES)

Since BRUCHMANN'S great work upon the prothallia of the European species of *Lycopodium* appeared in 1898, and probably before that date, numerous attempts have been made to find prothallia in America; but, so far as the writer has been able to determine, no successful searches have been reported.

While taking a correspondence course in botany with Professor CHARLES J. CHAMBERLAIN, he suggested that I avail myself of the opportunities afforded by my location in a *Lycopodium* region and make a thorough search for prothallia. In accordance with his suggestions and directions, the work was undertaken and has resulted in the finding of 21 prothallia and over 50 sporelings, representing 5 species: *L. clavatum*, *L. complanatum*, *L. annotinum*, *L. obscurum*, and *L. lucidulum*. It is also a pleasure to acknowledge my indebtedness to Dr. W. J. G. LAND for valuable suggestions.

The first specimen, that of *L. complanatum*, was found May 22, 1916. During the same month and in September of the same year, 6 more of this species, 8 of *L. clavatum*, 3 of *L. obscurum*, 2 of *L. annotinum*, and 1 of *L. lucidulum* were dug up, making 21 in all. Although the first sporeling bearing a foot, which indicated that a prothallium was recently present, was found on April 10, 1915, it was not until May 20, 1916, that a second was found 10 miles from it. During this interval approximately 150 days were spent crawling over the ground among and around the dense beds of adult sporophytes.

At first these tedious and futile efforts would seem to indicate that prothallia are rare. Yet, when it is noted that 17 of the 21 specimens were obtained from an area not more than 10 m. square, only about one-fourth of which was actually dug up; and furthermore, that as many as 6 were yielded by one small space 4 cm.

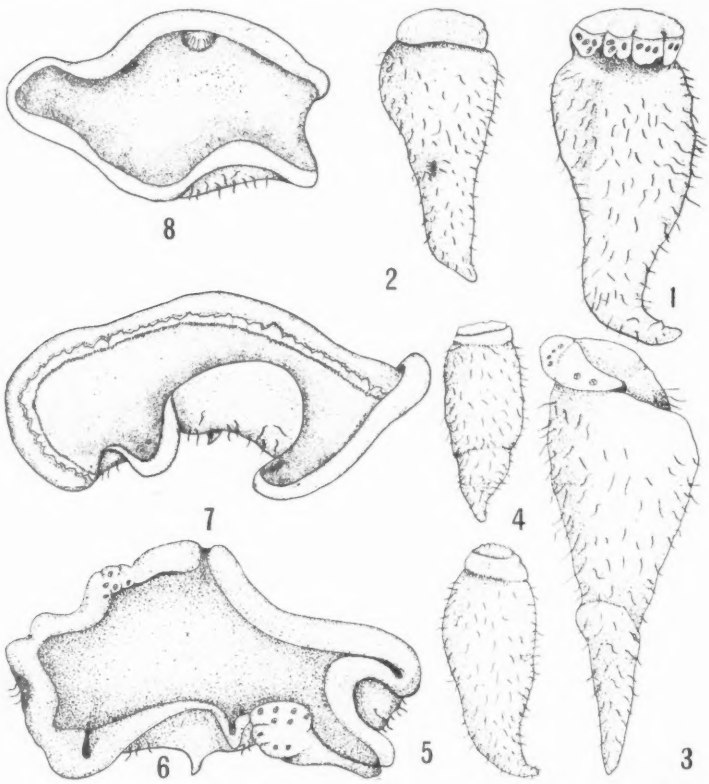
square, it seems probable that prothallia are rather abundant. The probability is even greater since the discoverer of these 21 prothallia was almost absolutely ignorant of the appearance of *Lycopodium* prothallia.

The discovery of the original locality was almost an accident. It is a place where one would least expect to find *Lycopodium* prothallia, at least those of *L. complanatum*, *L. clavatum*, and *L. annotinum*, since it is almost completely bare of adult sporophytes of these 3 species. I was wandering about looking for *Morchella*, when by chance I caught sight of an old sporeling of *L. clavatum*, growing in an exposed position between some wintergreen plants. An examination of the soil showed no prothallium. No adult sporophytes were within sight; but a sporeling is a good sign, and this encouragement led me to make the final successful search two days later.

Lying to the northwest of Marquette, Michigan, are a brewery and the remains of an old pesthouse. To the east of this pesthouse, about 700 m., is an open space bordering an open pasture on the north, a small wooded swamp on the east, and a wood composed of second growth poplar and maple on the west. A path runs to the northwest from the road which leads from Marquette to the pesthouse. Beside this path, at the distance indicated from the pesthouse, 18 of the 21 prothallia and most of the sporelings were found. The other prothallia and sporelings were found in two separate stations, the one half a mile, the other two miles from this one described.

The soil is mainly sand, covered in places by a very thin layer of humus; that within the edge of the wood is a black sandy loam. The specimens of *L. annotinum* and *L. lucidulum* shown in figs. 9 and 10 are the only ones which were found in the sandy loam where the ground is constantly shaded in summer. This specimen of *L. annotinum* is also enormously larger than the other prothallia, while that of *L. lucidulum* is decidedly the smallest. All the other specimens and most of the 50 sporelings were found in open, exposed, sandy places. The topography is uneven, rocks jutting up here and there between water-logged regions. Scattered about on the elevated regions are numerous small sandy knolls covered

partly by *Polytrichum*, and sometimes by a species of grass. In many spots these knolls are absolutely bare save for a few plants of *Polytrichum*. It was in these knolls that all but 2 of the 21 prothallia were found, as well as most of the sporelings.



FIGS. 1–8.—Fig. 1, prothallium of *L. complanatum*, first specimen found, ×5; figs. 2, 3, prothallia of *L. complanatum* (?), showing openings to the antheridia which form the lobes entirely, ×5; figs. 4, 5, prothallia of *L. obscurum* (?), crown is double, ×5; figs. 6–8, prothallia of *L. clavatum;* in fig. 6 the antheridial lobes are marked as in figs. 1 and 2; in fig. 7 the lobed edge is double; ×10.

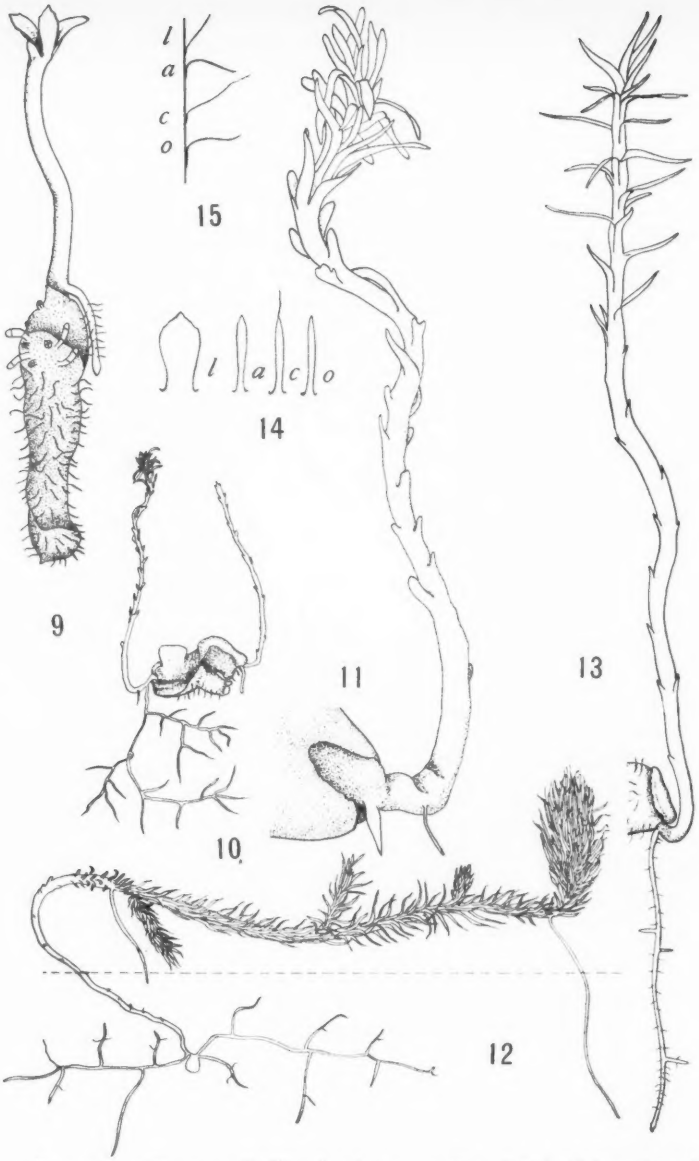
The frequent occurrence of sporelings and prothallia on these small, bare, exposed elevations suggests the idea that those spores which fall in such localities are first of all beaten into the ground

by the frequent rains; later, they are covered over by the shifting surface sand, and are finally conveyed to a favorable depth by the percolating soil water. No experiments were made to see whether this is the correct interpretation; but it was this idea which led me to investigate the knolls. This observation was heeded in all searches subsequent to the first successful one, and all yielded one or more prothallia.

So far as the elevation of the knolls is concerned, too much importance should not be placed upon it. The fact that they are elevated somewhat and exposed as they are to the air currents merely causes them to be covered by leaves less frequently than the lower or more wooded regions near them, and consequently they offer a more receptive landing to the spores which are transported thither. This idea is corroborated by the fact that many sporelings of *L. lucidulum* and *L. annotinum*, as well as 3 prothallia, were found in the middle of a trail which is frequently used by hunters. In these instances the spores were clearly trampled into the soil until they found the depth required for their growth into prothallia. I suspect that future observations may show that the prothallia of *L. annotinum* and *L. lucidulum* require more moisture for their development than the other species mentioned. The 3 specimens I found of these 2 species, although the surface indications were similar, grew in decidedly wetter places.

In searching for prothallia, soil was removed with forceps. This method was not very satisfactory, however, and 4 of the prothallia were broken or pierced by the forceps before they were seen. It would be better to remove the soil to a depth of 10 cm. and wash it through a coffee sieve. This would not only avoid the danger of damaging prothallia, but would increase the probability of finding young stages. The specimens grew at depths varying between 1 and 7 cm. The species growing nearest the surface is *L. lucidulum;* the one growing deepest is *L. obscurum*. In one hole 2 kinds, *L. clavatum* and *L. obscurum*, were seen within 2 cm. of each other.

The prothallia of *L. clavatum* and *L. annotinum* grow with the wrinkled side toward the surface, and the primary tubercle pointed downward; that of *L. lucidulum* grows erect and the sporeling



FIGS. 9–15.—Fig. 9, prothallium bearing a sporeling of *L. lucidulum*, the only specimen found, showing 4 paraphyses, archegonia, primary tubercle, rhizoids, and an enlargement made by foot of sporeling, ×6; fig. 10, prothallium of *L. annotinum* bearing 3 sporelings, smallest one just emerging from upper tip of lower lobe of gametophyte, natural size; fig. 11, detailed drawing of largest sporeling shown in fig. 10, ×3; fig. 12, old sporeling of *L. clavatum* with foot still present, having lost erect habit of younger sporelings and assumed trailing habit of adult, ×2; fig. 13, sporeling and portion of gametophyte of *L. obscurum*, rest of gametophyte being like corresponding portions of fig. 5, ×4.25; fig. 14, diagram showing leaf contour of sporelings of *L. lucidulum* (*l*), *L. annotinum* (*a*), *L. clavatum* (*c*), and *L. obscurum* (*o*); fig. 15, diagram showing general profile of leaves of sporelings, the leaf being directed upward in *L. lucidulum* (*l*), drooping in *L. annotinum* (*a*), directed upward but bearing bristle in *L. clavatum* (*c*), bending downward then upward in *L. obscurum* (*o*), leaving the stem almost at a right angle.

arises straight out of the end lying immediately beneath the surface of the soil. The habit of growth cannot be stated definitely for *L. obscurum* and *L. complanatum*. The specimen of *L. complanatum* shown in fig. 3 was found in the same position as the specimen of *L. obscurum* shown in fig. 13. All the other specimens of *L. obscurum* and *L. complanatum* which I collected were disturbed before I saw them, and consequently their exact position was not determined, except the one in fig. 1 which grew vertically. In size, the prothallia of figs. 4 and 5, are very similar to that of fig. 13. This resemblance in size, together with a common external contour, are my only reasons for assuming that these 3 specimens belong to the same species. But here arises a difficulty. Although the prothallium in fig. 3 is about twice as large as the adult prothallium of *L. obscurum* (fig. 13) bearing a sporeling, we find the two growing in exactly the same position. This may mean two things; either the prothallia I have classed with *L. complanatum*, except no. 1, might as readily belong to *L. obscurum*, or the prothallia of *L. complanatum* grow in various positions in regard to the directions of their axes. If the axis of a prothallium of *L. complanatum* can be shown to grow always in a vertical position, and if that of *L. obscurum* can be shown to grow always in a horizontal position, the identification of the two species will then become a very simple matter if care be taken while hunting for them. I can only regret that this important point must be left for future observation to settle.[1]

Adult sporophytes of *L. clavatum* and *L. annotinum* were rare in this immediate locality, only one small patch of each having been found. A few plants of *L. complanatum* were found. *L. obscurum* and *L. lucidulum* grow in small clumps throughout the region.

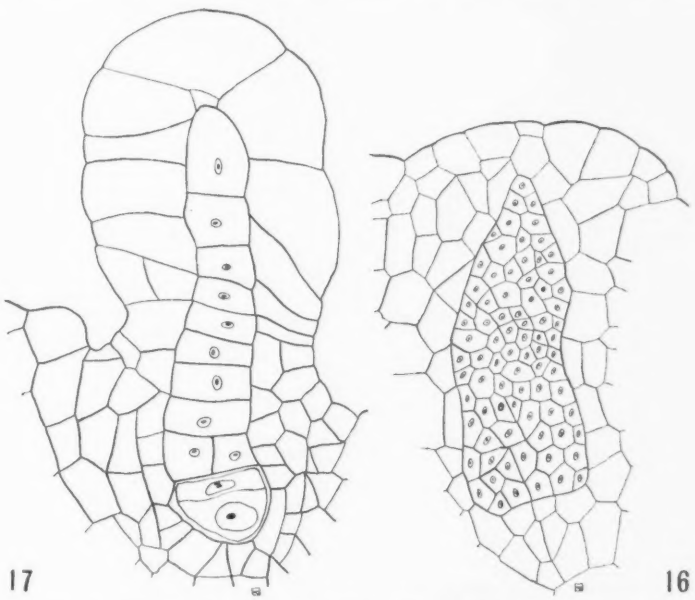
The 50 sporelings belong mainly to *L. clavatum*, but a few were found of all the other species mentioned in this paper except *L. complanatum*. Since the sporeling is the first guide in the

[1]On November 5, 1916, after this paper had gone to press, the writer found a large specimen of *L. obscurum* with sporeling attached. It was of the same color and shape as and grew horizontally like the prothallium labeled *L. complanatum* in fig. 3. This bears out the suggestion that all the prothallia classed with *L. complanatum*, except no. 1, very probably belong to *L. obscurum*.

search for prothallia as well as in their identification, I have given 2 diagrams in figs. 14 and 15 to show the specific differences between the juvenile leaves of the 4 species of sporelings which I obtained. If the descriptions of these 4 species as given in GRAY's *Manual* are followed carefully, the specific differences may readily be determined. The leaves of *L. clavatum* terminate in a bristle even in a stage as young as that sporeling of *L. annotinum* shown in figs. 10 and 11. I have not been able to separate sporelings of these 2 species at such a young stage or younger by any other character than by the presence or absence of the terminal bristle on the leaf. At such a stage the leaves have not yet assumed such very definite directions of growth as they do a little later, and as indicated in *a* and *c* of fig. 15. The sporeling of *L. obscurum* is easily identified by the 6 rows of leaves which early assume the characters peculiar to this species. Of course in the sporeling stage the leaves are fewer and more spreading than in the adult, so that a hurried examination would scarcely show the relationship between the two. I actually thought I was digging for a prothallium of *L. annotinum* while removing the soil from around the sporeling shown in fig. 13, and it was not until all the sand had been removed from among the rhizoids of the prothallium, and after the leaves had been examined under a lens, that I discovered my error. The sporeling of *L. lucidulum* cannot be confused in any way with the sporelings of any of the species I have mentioned. However, one may very readily believe that he is digging up a genuine one only to find that peculiar winged bud at the bottom of it. Yet, although I have experienced this disappointment a hundred or more times, through it I have observed that the sporelings bearing a foot grow just a trifle deeper in the ground. Generally the bud lies nearly or wholly on the surface, and unless it is rotted away, is readily seen. The sporeling of *L. lucidulum* looks exactly like one of the plants which grows from a bud, except that it has a distinct foot. Both bear leaves like an adult plant. Some of the plants in the vicinity are *Morchella*, *Polytrichum Acer*, *Populus*, *Pteris*, *Gaultheria*, *Rhus*, and *Polygala*.

The largest specimen of *L. annotinum* (fig. 10) measured 10 ×7 mm. It bore 3 sporophytes, one of which reached only 0.5

cm. above the ground, and another had just emerged from the prothallium. In color it resembled closely the gametophyte of *Botrychium virginianum*. The specimens of *L. clavatum* were much smaller and almost white in color. They varied in size from 6.5×5 mm. to 5×3 mm. The largest fragment measured 7×5 mm., and the smallest 2×1 mm. Figs. 6, 7, and 8 show the entire specimens. The fragments were more twisted and wrinkled than



FIGS. 16, 17.—Fig. 16, antheridium of *L. clavatum* in sperm mother cell stage, ×365; fig. 17, archegonium of *L. complanatum* immediately before neck canal cells break down, the lowest neck canal cell being double, ×365.

these, but both the fragments and the entire specimens showed a distinct crown on the border, which in one case (fig. 7) was double, the inner crown having an irregular outline. Each of the entire prothallia showed the primary tubercle very distinctly, near the middle, on the ventral side.

The prothallia of *L. complanatum* were all entire except one which had the lower portion broken off. Seven of these prothallia,

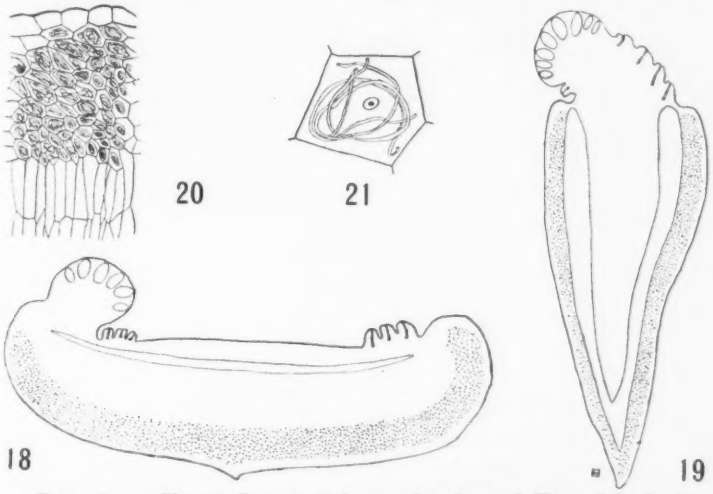
illustrated by figs. 1, 2, and 3, bore a single crown which was more or less lobed. In section (fig. 19) these lobes proved to be masses of antheridia. The crown on each of the 3 prothallia of *L. obscurum* (figs. 4, 5) was double. An accident to the sections prevented a study of the relation between the lobes and the location of the sex organs in these prothallia. The crown was unequal in every specimen of *L. complanatum* and *L. obscurum*, and the lobes appeared only at one side, as shown in fig. 3.

The one prothallium of my collection which may cause some readers to question is that of *L. lucidulum*. I have found 10 sporelings and only one prothallium, but this single prothallium fortunately has a sporeling attached. This fact alone would not serve to convince a botanist in doubt of its genuineness. However, there are 5 reasons why I am convinced of its nature. Fig. 9 shows these 5 points. They are (1) archegonia, (2) paraphyses, (3) rhizoids, (4) a primary tubercle, and (5) the sporeling arises from it like the sporeling of any recognized gametophyte; there is a foot, and a primary root originates at the point where the stem breaks through. Certainly all these features cannot be connected with a young plant of *L. lucidulum* of asexual origin or with an associated fungus growth.

This prothallium of *L. lucidulum*, which, like that of *L. obscurum*, I believe is new to botanists, shows some very interesting evolutionary points. The body is somewhat cylindrical, but not entirely so. It is somewhat more flattened longitudinally than the figure shows. In this figure the flat side is turned toward the reader. Near the middle it makes a short twist toward the left. In general outline it looks like a prothallium of *L. complanatum* in the making, but on the upper lobed region are 4 paraphyses. Such a feature suggests a transition stage between the *Phlegmaria* type of prothallium, as represented by *L. Selago*, and such a form as *L. complanatum*. The specimen contained no chlorophyll so far as I was able to determine.

Among the 50 sporelings gathered there was a variation in age between 1 and 5 years. Each season's growth above the soil could be determined definitely by the alternate regions of longer and shorter leaves on the species *L. clavatum* and *L. annotinum*.

Fig. 11 shows in detail the largest sporeling of *L. annotinum* growing from the prothallium represented in fig. 10. The sporeling of *L. clavatum* shown in Fig. 12 was one of the largest, and serves to indicate the size one may obtain still bearing the foot. It is very possible, even for one who has studied carefully the figures of *Lycopodium* prothallia given in the various papers upon the subject, to mistake other forms of tubers for them. The sex organs may be indistinct or undeveloped, so that identification by means of



FIGS. 18–21. Fig. 18, diagram of tissue regions in prothallium of *L. clavatum* (antheridia and archegonia indicated); fig. 19, diagram of median region of prothallium of *L. complanatum*, the fungus-infected region being indicated in this and fig. 18 by dotted shading; fig. 20, detailed sketch of fungus-infected region, showing its location beneath the epidermal tissue, ×75; fig. 21, single cell with endophytic fungus coiled within (fungus passes freely from one cell to another by piercing the cell wall), ×750.

them is impossible in such an instance. The number and variety of small tubers which grow in the soil of a wood are both large and confusing. Some of them are surprisingly similar in all outward appearances to small prothallia of *L. complanatum*. I do not doubt that I threw away some genuine specimens because they appeared to be tubers. I know that I retained some tubers and even sectioned them in paraffine because I thought they were prothallia.

To establish definitely whether the forms that I have collected are genuine prothallia of *Lycopodium*, therefore, I have drawn figs. 16, 17, 20, and 21 to show an antheridium, an archegonium in which the lowest of the neck canal cells is double, and 2 detailed sketches of the fungus-infected region. The diagrams in figs. 18 and 19 indicate the tissue regions shown by sections of 12 prothallia.

This article seeks to establish the fact that the prothallia of *Lycopodium* have been found in America; to make known to those botanists who may later desire to find prothallia for themselves, what the conditions were under which the specimens collected were found; and to announce the discovery of 2 new species of *Lycopodium* prothallia, namely, *L. obscurum* and *L. lucidulum*. Concerning the development and structure of the American *Lycopodium* gametophytes, the writer hopes to deal in a later paper.

MARQUETTE, MICH.

SIMILARITY IN THE EFFECTS OF POTASSIUM CYANIDE AND OF ETHER

W. J. V. OSTERHOUT

(WITH ONE FIGURE)

The writer has pointed out that typical anesthetics, such as ether, chloroform, and alcohol, produce a temporary decrease in permeability.[1] In view of the fact that anesthesia is looked upon by some as a form of asphyxiation, it seems desirable to investigate the manner in which permeability is affected by KCN, which not only acts as an anesthetic, but also inhibits oxidation to a remarkable degree.

The experiments here described were made in 1912, in connection with a series of experiments on anesthetics, of which a brief announcement has already appeared. Since then a paper by KREHAN[2] has been published which states that KCN produces a transitory increase of permeability which soon disappears. The writer is unable to confirm this statement, as will appear from the following account.

The experiments were made on tissues of *Laminaria Agardhii*. The permeability was measured by determining the electrical resistance in the manner described in previous publications.[3] The KCN employed was Kahlbaum's best, and the distilled water was prepared with especial care. A solution of KCN of the same conductivity as the sea water (about 0.381M) was prepared. This was added to the sea water and its effect on the tissues was observed. The following experiment will serve to illustrate the procedure.

A lot of tissue which had in sea water a resistance of 1140 ohms was placed in sea water to which had been added a solution of KCN 0.381M in sufficient quantity to make the concentration

[1] OSTERHOUT, W. J. V., The effect of anesthetics upon permeability. Science N.S. **37**: 111–112. 1913.

[2] Internat. Zeit. f. Phys. Chem. Biol. **1**: 189. 1914.

[3] OSTERHOUT, W. J. V., The permeability of protoplasm to ions and the theory of antagonism. Science N.S. **35**: 112–115. 1912.

0.01M.[4] The resistance rose rapidly to 1170 ohms, where it remained for 10 minutes, after which it began to fall. The results are given in table I and fig. 1.

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In KCN 0.01M in sea water	In sea water
0	1140	1080
10	1160	0
20	1160	0
30	1150	1070
110	1010	0
200	910	0
300	810	0
400	710	1070

All readings were taken at 14° C. or corrected to this temperature.

The resistance of the apparatus was 250 ohms; hence the resistance of the tissue (the net resistance) at the start was 1140−250=890 ohms. We may put the permeability as equal to the conductivity, or, for convenience, as equal to the conductance; hence the permeability was 1÷890=0.001124. The maximum net resistance was 1170−250=920 ohms, and the permeability was 1÷920=0.001087. Hence the loss of permeability was (0.001124−0.001087)÷0.001124=3.3 per cent.

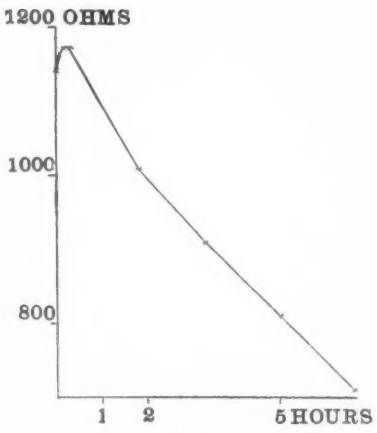


FIG. 1

Similar experiments were made with other concentrations from 0.002M up to 0.381M (solution of KCN without sea water). The results were irregular, and it is not possible to say

[4] This mixture had the conductivity of sea water. The sea water after the addition of the KCN was slightly alkaline to litmus. The hydrogen ion concentration of 0.01M KCN in sea water was 1.4×10^{-10} as determined by the gas chain. The alkalinity tends to make the rise of resistance less pronounced.

without numerous additional experiments at exactly what concentration the maximum decrease of permeability occurs. It seems doubtful whether it amounts to much more than 3 or 4 per cent at any concentration.

In KCN 0.381M[5] (without sea water) there was in some cases a rise in resistance, followed by a rapid fall, and in other cases the resistance did not rise, but fell from the start. It is probable, however, that in these cases there was a transitory rise which disappeared before the end of the first minute, at which time the first measurement was taken.

The experiments demonstrate that there is a temporary decrease of permeability instead of a temporary increase as described by KREHAN. At no concentration was a temporary increase of permeability observed. Whenever the permeability began to increase, it continued to increase steadily until the tissue was dead. The concentrations employed ranged from 0.002M to 0.381M. It may be added that the method of plasmolysis, which was employed by KREHAN, cannot be relied upon to give as accurate measurements of permeability as the determination of electrical resistance.

If tissue be allowed to remain in KCN until the resistance has fallen about 100 ohms, it will often completely regain its original resistance on being transferred to sea water. But if the resistance be allowed to fall much beyond this, recovery is usually incomplete and the greater the fall of resistance (beyond the point where complete recovery is possible) the less the recovery.

The concentrations of KCN necessary to produce a decrease of permeability are very much smaller than the corresponding concentrations of ether, chloroform, and alcohol. This accords with the fact that it also takes less KCN to produce narcosis. The period of decreased permeability cannot be prolonged as much by means of KCN as by means of the other anesthetics mentioned. This agrees with the fact that organisms can be kept longer under

[5] The hydrogen ion concentration was 7×10^{-13} as measured by the gas chain. This is sufficiently alkaline to cause a considerable fall in resistance (cf. Jour. Biol. Chem. **19**: 335. 1914). The concentration of KCN was determined by weighing out the requisite amount, but owing to the presence of alkali it was really less than 0.381M.

narcosis without injury by means of ether, chloroform, and alcohol than by means of KCN.

The fact that KCN resembles typical anesthetics (such as ether and chloroform) in producing a temporary decrease in permeability does not, in the opinion of the writer, show that anesthesia is a form of asphyxiation. It seems quite probable that the decrease of permeability and the anesthesia produced by KCN have connection with its effect on oxidation.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

CURRENT LITERATURE

NOTES FOR STUDENTS

Oenothera genetics.—HERIBERT-NILSSON[1] discusses the data from his studies of *Oenothera Lamarckiana*, suggesting what he calls a Mendelian interpretation of the mutating tendency of this species. The character with which he worked was the red pigmentation found in the leaf nerves of some of his plants and absent in others. He concludes that the red-nerved and white-nerved plants form a distinct discontinuous variation; that the white-nerved plants are pure recessives and when selfed or intercrossed produce only white-nerved plants; that a homozygous dominant is not formed, and that therefore a strain of pure red-nerved plants cannot be produced, but all red-nerved plants when selfed or intercrossed will produce some white-nerved plants. Finding the average ratio of red-nerved to white-nerved plants in *O. Lamarckiana* and most of its "mutants" to be 2.68:1, or nearly 3:1, instead of 2:1 as would be expected when no positive homozygotes are formed, he adopts the explanation proposed by WILSON in explaining the work of CUÉNOT with yellow mice. According to this explanation, most of those positive female gametes which would normally be fertilized by positive male gametes, but which for some reason cannot be so fertilized, are fertilized by recessive male gametes. This would produce an average ratio of the red-nerved to white-nerved plants a little lower than would be the case under normal genetic behavior, thus accounting for a ratio of 2.68:1 instead of 3:1. It should not be forgotten, however, that the work of CASTLE removed the necessity for this interpretation in the case of yellow mice, and thus lessened its value as an interpretation of this sort of deviation from expected ratios.

In "*gigantea*" (the *gigas* type) the author interprets the observed ratios as modifications of 3:1, 15:1, 63:1, and 255:1, and concludes that in this type the red-nervedness is probably produced by any one of four factors. He also finds that the factor or factors for red leaf nerves affects other morphological and physiological characters of the plant.

Having thus striven for a Mendelian interpretation of the behavior of red vs. white nerves, the author presents his observations on the mutation ratios of *O. Lamarckiana* and its mutants, or, as he calls them, "Kombinante," and suggests the following explanation of the mutating tendency of this species. *O. Lamarckiana* is dependent upon a number of groups of multiple factors, the majority of which cannot be produced in a homozygous dominant condition,

[1] HERIBERT-NILSSON, N., Die Spaltungserscheinungen der *Oenothera Lamarckiana*. Lunds Univ. Årsskrift **12**:4–131. 1915.

and the various mutants are plants which result when one or more of these groups are in a homozygous recessive condition. This might be represented graphically thus: *O. Lamarckiana* = *Aa Aa Aa aa; Bb Bb bb bb; Cc Cc cc cc*, etc., where in every group at least one of the factors would be present in the positive condition. A mutant = *aa aa aa aa; Bb Bb bb bb; Cc Cc cc cc*, etc., where in at least one of the groups none of the factors are present in the positive condition. This interpretation is thought to explain the occurrence of different ratios of mutation, for if there were 4 such independent multiple factors for the *Lamarckiana* character, a given mutant dependent upon the absence of all these positive factors would occur in the following percentage: 1.2 per cent when all of the 4 factors are heterozygous; 3.7 per cent when only 3 of the 4 factors are heterozygous; 11.1 per cent when only 2 of the 4 factors are heterozygous; 33.3 per cent when only 1 of the 4 factors is heterozygous.

At several points in his paper the author points out that since different strains of *O. Lamarckiana* yield different series of mutants it cannot be an elementary species, as DEVRIES claims, but must be a group of elementary species, the free intercrossing of which makes possible the production of the mutants by ordinary Mendelian segregations. The assumption of extensive linkage of characters, of the occurrence of heterogamy (that is, the transmission of hereditary characters through the sperms differing from those possessed by the eggs of the same individual), and the assumption that one sort of sperm may hinder the activities of another sort of sperm, are not in strict accord with the author's claim that he has given a Mendelian interpretation of *Oenothera* genetics.—BEN C. HELMICK.

Mutation in Matthiola annua, a "Mendelizing" species.—In a preliminary paper under the foregoing title FROST[2] has published certain conclusions in regard to the origin of Mendelian dominants which are sure to arouse no little interest. Until the full account appears it will be impossible to judge of the validity of FROST'S interpretation of his discoveries, but the discoveries themselves are obviously of prime importance, interpret them however we may. According to his own view, he has observed the origin by mutation of 8 different dominant Mendelian varieties from a single strain of stocks. To show that this would be a discovery of the highest theoretical significance, it is only needful to point out that similar evidence is extremely meager, and in practically every case not as well attested as one might wish. The list of new dominants which have arisen by mutation is practically exhausted when we have mentioned KEEBLE'S giant *Primula* and COLLINS' albinistic maize, for the case of GATES' *Oenothera rubricalyx* is still in dispute.

FROST states that the individual mutations of his *Matthiola* cultures obviously are not extracted recessives, but heterozygous dominants; that they seem to be due to definite changes in the germ plasm distinct from the recombi-

[2] FROST, HOWARD B., Mutation in *Matthiola annua*, a "Mendelizing" species. Amer. Jour. Bot. **3**:377–383. 1916.

nations involved in ordinary Mendelian phenomena; that the mutative changes concern various characteristics of the plant, but that the factor for each new type is regularly inherited as a unit, sometimes showing linkage with another factor pair, so that we may suppose, in some cases at least, that the essential change is limited to a portion of one chromosome. The very first test of these conclusions would demand that the mutations reproduce the mutational type in 75 per cent of their progeny in the first generation, and that 25 per cent of the progeny be homozygous dominants. This condition apparently is satisfied in the case of only 1 mutation of the 8, and until the data appear we have no basis for an independent judgment as to whether the progenies of the second generation were large enough to prove the point at issue. Except from this one mutation, no homozygous mutational type has segregated from any of the supposed heterozygous dominants. In the mind of one who is familiar with the group of the evening primroses a suspicion naturally arises that FROST's mutations are not Mendelian at all, but that they show the type of behavior familiar in *Oenothera lata* DeVries, and recently discovered in mutations from *O. stenomeres* and *O. pratincola*. These mutations always give progenies consisting of a mixture of the parental and mutational types. In the case of *O. lata* the cytological explanation is now so well known as hardly to require comment; it certainly suggests that a cytological examination of the *Matthiola* mutations would not be amiss. Reciprocal crosses between the mutational and parental types might also throw light on the possible analogy between the evening primroses and stocks, for in such types as *Oenothera lata* mutational characters are carried only by part of the female gametes, and by none of the male gametes. All that FROST tells about the *Matthiola* mutations so exactly parallels what is found in *Oenothera* that one can hardly refrain from suggesting, in the absence of data supporting his own interpretation, that instead of discovering new Mendelian dominants he has found in a widely distant group some of the perplexing phenomena which critics of the mutation theory persist in regarding as peculiar to *Oenothera*. More and more facts are coming to light in groups other than *Oenothera* which do not fall into line according to Mendelian expectations. As an example of what looks like mutation in the DeVriesian sense, one thinks of the rogues of peas, investigated by BATESON; as an example of matroclinic, non-segregating hybrids, quite comparable to those of *Oenothera*, we have the cases in *Primula*, recently reported by PELLEW and DURHAM. If the type of heredity shown by *Oenothera lata* were found to apply to the mutations of *Matthiola*, it would be almost as interesting as the discovery of new Mendelian dominants.—H. H. BARTLETT.

Respiration in succulents.—That succulent plants exhibit peculiarities in their respiratory processes and periodic changes in acidity with light and darkness has been known for a long time. RICHARDS[3] has investigated these

[3] RICHARDS, HERBERT M., Acidity and gas interchange in cacti. Carnegie Inst., Washington, Publication no. 209. pp. 107. 1915.

periodic acidity changes and the respiratory ratios in cacti, a group heretofore not sufficiently studied. Extensive work has been done, principally with *Opuntia versicolor*, with results in general agreement with what is already known regarding respiration in succulents. The paper presents a large mass of data, and considers the influence of light, temperature, oxygen supply, and wounding on the acidity of the tissues, and devotes considerable space to the relation of acidity, light, temperature, oxygen, etc., to the rate and ratio of gas interchanges. The production of the acid, chiefly malic acid in cacti, is thought to be due to lack of oxygen in the tissues, owing to anatomical structures which, to restrict transpiration, restrict the other gas exchanges as well. During the night the acid accumulates, because the chief factors capable of causing deacidification, namely, light, high temperature, prolonged darkness, and unusually high oxygen pressures, are absent.

The true respiratory quotient for cacti is low, and can be measured accurately only when acidity is stationary or rising. For during falling acidity, the approach of the ratio to the typical ratio, unity, is not real, because the increased CO_2 is furnished merely by the decomposition of the acid, which is not considered a respiratory process. Some of the minor points brought out are that while CO_2 production closely parallels rise and fall of temperature, it lags behind by about an hour, maximum and minimum CO_2 production being reached about an hour later than maximum and minimum temperature; and that total acidity increases more rapidly than the acid concentration of the juice. This is reasonably traced to greater hydration of the colloids in the presence of the acids, and to an increased osmotic pressure in the cell sap leading to greater turgidity.

The main point of interest to physiologists is the interpretation of the phenomena, which differs somewhat from that of NATHANSOHN, who looked upon the breaking down of the acids by day as a completion of the respiratory process at a time when CO_2 could be used in photosynthesis. This view makes the CO_2 production during deacidification a source of respiratory energy, and at the same time of great biological significance in conserving the raw materials for photosynthesis. RICHARDS considers the acid the end product of respiration rather than an intermediate product. The breaking down of the acid by day is due chiefly to light, aided by the accompanying high temperature. The reaction is photolytic and not respiratory, probably takes place in the cell sap, and therefore probably yields its energy not in connection with the living protoplasm. He points out that CO_2 production during deacidification may be so rapid as to exceed photosynthetic use of the gas, and states that "whatever of energy there may be from the final oxidation of the acid outside the sphere of protoplasmic activity is simply the result of anatomical peculiarities of the plant, the advantages of which may well outweigh this loss."

The whole problem of acidity and gas exchange under life conditions is necessarily a very complex one because so many variable factors are involved,

and a careful reading of the paper emphasizes this fact. Conclusions must therefore be drawn with considerable care.—CHARLES A. SHULL.

Insects and plant diseases.—Although both botanists and entomologists have realized for a long time that insects are carriers of organisms of plant diseases, very little attention has been given to the study of the subject. However, there is now a tendency to take up this line of investigation. Four papers have come to the reviewer's desk recently.

A paper by RAND[4] on the dissemination of the bacterial wilt of cucurbits follows out a suggestion given by ERWIN F. SMITH and produces evidence indicating that this leaf-eating cucumber beetle (*Diabrotica vittata*) is both the summer and the winter carrier of the *Bacillus tracheiplilus* which causes the wilt of cucumbers and other cucurbits.

In a later paper by RAND and ENLOWS,[5] the authors not only confirm the conclusions given by RAND in the first paper, but also include the 12-spotted cucumber beetle (*D. duodecimpunctata*) as an important summer carrier of this organism. In experiments by the same authors, the squash bug (*Anasa tristis*), the flea beetle (*Crepidodera cucumeris*), the melon aphis (*Aphis gossypii*), and the 12-spotted lady beetle (*Epilachna borealis*) did not transmit the disease.

Another paper by HYSLOP[6] on *Triphleps insidiosus* and corn rots gives conclusive evidence that this insect is the carrier of the fungi causing ear rots. In view of the fact that this insect has been considered beneficial since about 1881, HYSLOP's studies are of more than ordinary interest.

A fourth paper by STEWART and LEONARD[7] records their results with a number of experiments and comes to the conclusion "that all of the sucking bugs found in the nursery are of more or less importance in producing fire blight infections and must be considered *tout ensemble*. The relative importance of each species is difficult to determine. By virtue of their method of feeding and prevalence during each season, certain species are undoubtedly more destructive than others. On the other hand, under special conditions when a certain species is found in large numbers it may become of considerable importance. Usually the tarnished plant bug is more injurious than the leaf-hopper from the fact that the greater percentage of leaf-hopper punctures occur in the leaf tissue."—MEL T. COOK.

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[4] RAND, F. V., Dissemination of bacterial wilt of cucurbits. Jour. Agric. Research **5**:257–260. 1915.

[5] RAND, F. V., and ENLOWS, ELLA, M., Transmission and control of bacterial wilts of cucurbits. Jour. Agric. Research **6**:417–434. 1916.

[6] HYSLOP, J. A., *Triphleps insidiosus* as the probable transmitter of corn ear rot (*Diplodia* sp. *Fusarium*). Jour. Econ. Entomology **9**:435–437. 1916.

[7] STEWART, V. B., and LEONARD, M. D., Further studies on the rôle of insects in the dissemination of fire blight bacteria. Phytopath. **6**:152–158. 1916.

Taxonomic notes.—BAILEY[8] has published in advance some of the changes in nomenclature that will appear in the *Standard Cyclopedia of Horticulture*. The changes selected for publication involve the names of 100 species and varieties, and some of the changes affect North American species. For example, the retention of *Malus* in *Pyrus* involves changes in 24 names; while a new interpretation of *Statice* as contrasted with *Limonium* calls for changes in 43 names. The author pays his respects to a certain type of taxonomic work as follows: "It has been the desire, in the compilation of the cyclopedia, to accept new generic limitations with caution. The temper of the present times is to find differences, as opposed to the tendency of the immediately preceding workers to find agreements. The analytic intention is the mark of systematic work in this generation, as the synthetic intention was the mark of the past generation. There is reason to expect a return from the method of disunion to the method of relationships; and as a work designed for the use of horticulturalists, who cannot be skilled in bibliography and pedantry, should be conservative, I have thought it best, so far as possible, to avoid unnecessary and fantastic sub-divisions."

CONARD[9] has revived the discussion concerning certain generic names of our water lilies. With the help of even the more conservative manuals, we were accustoming ourselves to say *Castalia* when we thought of *Nymphaea*, and to say *Nymphaea* when we thought of *Nuphar*. Now CONARD has shown that the valid generic name for the white water lilies is *Nymphaea* after all, and for the yellow pond lilies is *Nuphar*.

FERNALD[10] has discussed the species of *Sabatia* usually recognized as occurring in New England, and has described a new species (*S. Kennedyana*) occurring in Massachusetts and Rhode Island.—J. M. C.

Life cycles of bacteria.—LÖHNIS and SMITH,[11] in a preliminary communication, present some of their conclusions from a study of 42 strains of bacteria. All of these strains showed life cycles "not less complicated than those of other microorganisms"; and the authors are inclined to believe that this may be true of all species of bacteria. The forms studied live alternately in an organized and in an amorphous stage, the latter being called a "symplastic" stage, because in this stage the separate cells undergo "a thorough mixing." From this "symplasm" new individual cells arise in various ways. In all cases what are called "regenerative units" become visible, which increase in size, and

[8] BAILEY, L. H., Nomenclatorial transfers. Rhodora **18**:152–160. 1916.

[9] CONARD, HENRY S., *Nymphaea* and *Nuphar* again. Rhodora **18**:161–164. 1916.

[10] FERNALD, M. L., The genus *Sabatia* in New England. Rhodora **18**:145–152. *pl. 121*. 1916.

[11] LÖHNIS, F., and SMITH, N. R., Life cycles of the bacteria. Jour. Agric. Research **6**:675–702. *pls. 1–7. fig. 1*. 1916.

later "either by germination or by stretching become cells of normal shape." A direct union of two or more individual cells was also observed, the significance of which was not studied. The authors state that "the life cycle of each species of bacteria studied is composed of several subcycles, showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions." It is obvious that if such life cycles are established for bacteria in general, a new field is opened up in bacteriology.—J. M. C.

Cane sugar and translocation.—BOYSEN-JENSEN[12] concludes that cane sugar plays an important rôle in the germination of pea seeds. In the first stages of germination the cane sugar present in the ungerminated seed is used both as building and respiratory material, as is evident from its reduction in amount during the first few days of the process. In the second stage of germination cane sugar is the translocation form of the starch, as is shown by the following facts: (1) there is a higher concentration in the cotyledons than in the axillary organs; (2) the concentration rises with time in the isolated cotyledons and falls in the isolated axillary organs; (3) only inconsiderable amounts of reducing sugars are present in the cotyledons. The author cites several investigations showing the frequent appearance of cane sugar as the translocation form of starch, and concludes by saying that either monosaccharides or disaccharides may be translocation forms of starch, depending upon the character of the plant part.—WILLIAM CROCKER.

Cabbage yellows.—GILMAN[13] has investigated this disease and the relation of temperature to its occurrence. It is a wilt disease caused by *Fusarium conglutinans*, which is a facultative parasite living in the soil, and under certain conditions becoming destructive to cabbage. It has a high optimum temperature and is very resistant to drying. Inoculation experiments were largely successful in inducing the disease, the failures being due obviously to variations in the virulence of the cultures and in the susceptibility of the host. Control plants remained entirely free from the disease. The appearance of the characteristics symptoms is dependent upon a temperature of 17–22° C. or above, lower temperatures preventing the occurrence of the disease. Field observations through three seasons confirmed this relation between the occurrence of the disease and high temperature.—J. M. C.

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[12] BOYSEN-JENSEN, P., Vorkommen, Bedeutung, und Bildung des Rohrzuckers bei der Keimung von *Pisum sativum*. Jahrb. Wiss. Bot. **56**:431–446. 1915. PFEFFER'S Festschrift.

[13] GILMAN, J. C., Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. **3**:25–84. *pls. 2. figs. 21.* 1916.

Rot of potato tubers.—HAWKINS,[14] continuing his studies on the effect of various fungi on their hosts, has investigated the effect of *Fusarium oxysporum*, *F. radicicola*, and *F. coeruleum* on the sugar content, both sucrose and reducing sugar, pentosans, methyl pentosans, galactans, dry matter, starch, and crude fiber of the potato tuber. The crude fiber content of the tubers was not reduced, starch and methyl pentosans were not affected appreciably, while the content of the other substances was reduced. It is interesting from the point of view of resistance to fungus invasion that the least digestible forms occur in greatest proportions in the skin and cortical regions of the tuber. *Fusarium oxysporum* and *F. radicicola* were found to secrete sucrase, maltase, xylanase, and diastase. The diastase, like the malt diastase that BROWN and MORRIS worked with, is incapable of attacking ungelatinized potato starch.—GEORGE K. K. LINK.

Phytoplankton of the oriental tropics.—OSTENFELD[15] has published a list of the phytoplankton of one of the straits of the Malay Archipelago. The list is based chiefly upon a large collection of drawings made by P. TH. JUSTESEN in 1909 and 1910, while residing at one of the small military stations in the Dutch Indies. The list includes 100 species, the largest group being the diatoms, with 56 species representing 23 genera. The Peridiniales constitute the other large group, including 40 species in 11 genera, the largest genera being *Ceratium* with 17 species, and *Peridinium* with 12 species. The general character of the plankton is said to be that of a "tropical neritic plankton," very much like the plankton examined by CLEVE and OSTENFELD from the Malay Archipelago and the Gulf of Siam.—J. M. C.

Branched prothallia.—Miss WUIST[16] has investigated the early stages of the gametophytes of the Polypodiaceae in reference to branching, subjecting them to various culture conditions. She observed branching in cultures of 15 species representing 9 genera. Branching, which was both dichotomous and monopodial, was not a response to any one type of culture medium, but appeared on distilled water, on soil, and on various nutrient solutions. Branches did not appear at any definite period in the life history of the gametophyte, but were formed by any cell of the filament, by divisions of the last cell of the filament, and from the margin and apex of the expanded portion of the prothallium. The author has concluded that a definite relation exists between branching and nutrition.—J. M. C.

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[14] HAWKINS, LON A., Effect of certain species of *Fusarium* on the composition of the potato tuber. Jour. Agric. Research **6**:184–196. 1916.

[15] OSTENFELD, C. H., A list of phytoplankton from the Boeten Strait, Celebes. Dansk Bot. Arkiv **2**:no. 4. pp. 18. *figs. 10.* 1915.

[16] WUIST, ELIZABETH D., Branched prothallia in the Polypodiaceae. Bull. Torr. Bot. Club **43**:365–383. *figs. 15.* 1916.